



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/AU97/00729  <b>(22) International Filing Date:</b> 31 October 1997 (31.10.97)  <b>(30) Priority Data:</b> PO 3384 1 November 1996 (01.11.96) AU PO 5117 14 February 1997 (14.02.97) AU  <b>(71) Applicant (for all designated States except US):</b> THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH [AU/AU]; Royal Parade, Parkville, VIC 3052 (AU).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> HILTON, Douglas, J. [AU/AU]; 244 Research Road, Warandyte, VIC 3113 (AU). ALEXANDER, Warren, S. [AU/AU]; 13 Park Street, Moonee Ponds, VIC 3039 (AU). VINEY, Elizabeth, M. [AU/AU]; 7 Wallara Crescent, Bundoora, VIC 3083 (AU). WILSON, Tracy, A. [AU/AU]; 26 Fortuna Avenue, North Balwyn, VIC 3104 (AU). RICHARDSON, Rachael, T. [AU/AU]; 24 Robinson Street, East Brighton, VIC 3187 (AU). STARR, Robyn [AU/AU]; 280 Drummond Street, Carlton, VIC 3053 (AU). NICHOLSON, Sandra, E. [AU/AU]; 66 Alma Terrace, Newport, VIC 3015 (AU). MBYCALF, Donald [AU/AU]; 268 Union Road,		Balwyn, VIC 3103 (AU). NICOLA, Nicos, A. [AU/AU]; 56 Churchill Street, Mont Albert, VIC 3127 (AU).  <b>(74) Agents:</b> HUGHES, E., John, L. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).  <b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> THERAPEUTIC AND DIAGNOSTIC AGENTS CAPABLE OF MODULATING CELLULAR RESPONSIVENESS TO CYTOKINES		
<b>(57) Abstract</b>  The present invention relates generally to therapeutic and diagnostic agents. More particularly, the present invention provides therapeutic molecules capable of modulating signal transduction such as but not limited to cytokine-mediated signal transduction. The molecules of the present invention are useful, therefore, in modulating cellular responsiveness to cytokines as well as other mediators of signal transduction such as endogenous or exogenous molecules, antigens, microbes and microbial products, viruses or components thereof, ions, hormones and parasites.		

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## THERAPEUTIC AND DIAGNOSTIC AGENTS CAPABLE OF MODULATING CELLULAR RESPONSIVENESS TO CYTOKINES

**FIELD OF THE INVENTION**

5 The present invention relates generally to therapeutic and diagnostic agents. More particularly, the present invention provides therapeutic molecules capable of modulating signal transduction such as but not limited to cytokine-mediated signal transduction. The molecules of the present invention are useful, therefore, in modulating cellular responsiveness to cytokines as well as other mediators of signal transduction such as endogenous or exogenous molecules, antigens, microbes  
10 and microbial products, viruses or components thereof, ions, hormones and parasites.

Bibliographic details of the publications referred to in this specification by author are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined after the bibliography. A  
15 summary of the SEQ ID NOs is given in Table 1.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other  
20 integer or group of integers.

**BACKGROUND OF THE INVENTION**

Cells continually monitor their environment in order to modulate physiological and biochemical  
25 processes which in turn affects future behaviour. Frequently, a cell's initial interaction with its surroundings occurs *via* receptors expressed on the plasma membrane. Activation of these receptors, whether through binding endogenous ligands (such as cytokines) or exogenous ligands (such as antigens), triggers a biochemical cascade from the membrane through the cytoplasm to the nucleus.

30

Of the endogenous ligands, cytokines represent a particularly important and versatile group.

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Cytokines are proteins which regulate the survival, proliferation, differentiation and function of a variety of cells within the body [Nicola, 1994]. The haemopoietic cytokines have in common a four-alpha helical bundle structure and the vast majority interact with a structurally related family of cell surface receptors, the type I and type II cytokine receptors [Bazan, 1990; Sprang, 5 1993]. In all cases, ligand-induced receptor aggregation appears to be a critical event in initiating intracellular signal transduction cascades. Some cytokines, for example growth hormone, erythropoietin (Epo) and granulocyte-colony-stimulating factor (G-CSF), trigger receptor homodimerisation, while for other cytokines, receptor heterodimerisation or heterotrimerisation is crucial. In the latter cases, several cytokines share common receptor subunits and on this basis 10 can be grouped into three subfamilies with similar patterns of intracellular activation and similar biological effects [Hilton, 1994]. Interleukin-3 (IL-3), IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) use the common  $\beta$ -receptor subunit ( $\beta_c$ ) and each cytokine stimulates the production and functional activity of granulocytes and macrophages. IL-2, IL-4, IL-7, IL-9, and IL-15 each use the common  $\gamma$ -chain ( $\gamma_c$ ), while IL-4 and IL-13 share an 15 alternative  $\gamma$ -chain ( $\gamma'_c$  or IL-13 receptor  $\alpha$ -chain). Each of these cytokines plays an important role in regulating acquired immunity in the lymphoid system. Finally, IL-6, IL-11, leukaemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin (CT) share the receptor subunit gp130. Each of these cytokines appears to be highly pleiotropic, having effects both within and outside the haemopoietic system [Nicola, 20 1994].

In all of the above cases at least one subunit of each receptor complex contains the conserved sequence elements, termed box1 and box2, in their cytoplasmic tails [Murakami, 1991]. Box1 is a proline-rich motif which is located more proximal to the transmembrane domain than the 25 acidic box 2 element. The box-1 region serves as the binding site for a class of cytoplasmic tyrosine kinases termed JAKs (Janus kinases). Ligand-induced receptor dimerisation serves to increase the catalytic activity of the associated JAKs through cross-phosphorylation. Activated JAKs then tyrosine phosphorylate several substrates, including the receptors themselves. Specific phosphotyrosine residues on the receptor then serve as docking sites for SH2-containing 30 proteins, the best characterised of which are the signal transducers and activators of transcription (STATs) and the adaptor protein, shc. The STATs are then phosphorylated on tyrosines,

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probably by JAKs, dissociate from the receptor and form either homodimers or heterodimers through the interaction of the SH2 domain of one STAT with the phosphotyrosine residue of the other. STAT dimers then translocate to the nucleus where they bind to specific cytokine-responsive promoters and activate transcription [Darnell, 1994; Ihle, 1995; Ihle, 1995]. In a  
5 separate pathway, tyrosine phosphorylated shc interacts with another SH2 domain-containing protein, Grb-2, leading ultimately to activation of members of the MAP kinase family and in turn transcription factors such as fos and jun [Sato, 1993; Cutler, 1993]. These pathways are not unique to members of the cytokine receptor family since cytokines that bind receptor tyrosine kinases also being able to activate STATs and members of the MAP kinase family [David, 1996;  
10 Leaman, 1996; Shual, 1993; Sato, 1993; Cutler, 1993].

Four members of the JAK family of cytoplasmic tyrosine kinases have been described, JAK1, JAK2, JAK3 and TYK2, each of which binds to a specific subset of cytokine receptor subunits. Six STATs have been described (STAT1 through STAT6), and these too are activated by  
15 distinct cytokine/receptor complexes. For example, STAT1 appears to be functionally specific to the interferon system, STAT4 appears to be specific to IL-12, while STAT6 appears to be specific for IL-4 and IL-13. Thus, despite common activation mechanisms some degree of cytokine specificity may be achieved through the use of specific JAKs and STATs [Thierfelder, 1996; Kaplan, 1996; Takeda, 1996; Shimoda, 1996; Meraz, 1996; Durbin, 1996].

20 In addition to those described above, there are clearly other mechanisms of activation of these pathways. For example, the JAK/STAT pathway appears to be able to activate MAP kinases independent of the shc-induced pathway [David, 1995] and the STATs themselves can be activated without binding to the receptor, possibly by direct interaction with JAKs [Gupta,  
25 1996]. Conversely, full activation of STATs may require the action of MAP kinase in addition to that of JAKs [David, 1995; Wen, 1995].

While the activation of these signalling pathways is becoming better understood, little is known of the regulation of these pathways, including employment of negative or positive feedback  
30 loops. This is important since once a cell has begun to respond to a stimulus, it is critical that the intensity and duration of the response is regulated and that signal transduction is switched

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off. It is likewise desirable to increase the intensity of a response systemically or even locally as the situation requires.

In work leading up to the present invention, the inventors sought to isolate negative regulators of signal transduction. The inventors have now identified a new family of proteins which are capable of acting as regulators of signalling. The new family of proteins is defined as the suppressor of cytokine signalling (SOCS) family based on the ability of the initially identified SOCS molecules to suppress cytokine-mediated signalling. It should be noted, however, that not all members of the SOCS family need necessarily share suppressor function nor target solely cytokine mediated signalling. The SOCS family comprises at least three classes of protein molecules based on amino acid sequence motifs located N-terminal of a C-terminal motif called the SOCS box. The identification of this new family of regulatory molecules permits the generation of a range of effector or modulator molecules capable of modulating signal transduction and, hence, cellular responsiveness to a range of molecules including cytokines. The present invention, therefore, provides therapeutic and diagnostic agents based on SOCS proteins, derivatives, homologues, analogues and mimetics thereof as well as agonists and antagonists of SOCS proteins.

## SUMMARY OF THE INVENTION

The present invention provides *inter alia* nucleic acid molecules encoding members of the SOCS family of proteins as well as the proteins themselves. Reference hereinafter to "SOCS" encompasses any or all members of the SOCS family. Specific SOCS molecules are defined numerically such as, for example, SOCS1, SOCS2 and SOCS3. The species from which the SOCS has been obtained may be indicated by a preface of a single letter abbreviation where "h" is human, "m" is murine and "r" is rat. Accordingly, "mSOCS1" is a specific SOCS from a murine animal. Reference herein to "SOCS" is not to imply that the protein solely suppresses cytokine-mediated signal transduction, as the molecule may modulate other effector-mediated signal transductions such as by hormones or other endogenous or exogenous molecules, antigens, microbes and microbial products, viruses or components thereof, ions, hormones and parasites. The term "modulates" encompasses up-regulation, down-regulation as well as

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maintenance of particular levels.

One aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative,  
5 homologue, analogue or mimetic thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein comprises a SOCS box in its C-terminal region

Another aspect of the present invention provides a nucleic acid molecule comprising a sequence  
10 of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein comprises a SOCS box in its C-terminal region and a protein:molecule interacting region.

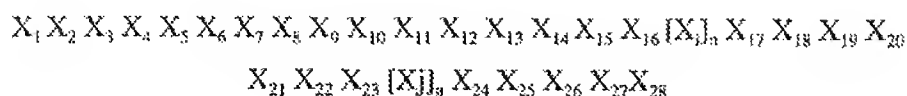
15 Yet another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein comprises a C-terminal region and a protein:molecule interacting region located in a region N-terminal of the  
20 SOCS box,

Preferably, the protein:molecule interacting region is a protein:DNA or protein:protein binding region.

25 Still a further aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein comprises a SOCS box in its C-terminal region and one or more of an SH2 domain, WD-40 repeats or  
30 ankyrin repeats N-terminal of the SOCS box.

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Even still a further aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein  
 5 comprises a SOCS box in its C-terminal region wherein the SOCS box comprises the amino acid sequence:



10

wherein:  $X_1$  is L, I, V, M, A or P;

$X_2$  is any amino acid residue;

$X_3$  is P, T or S;

$X_4$  is L, I, V, M, A or P;

15

$X_5$  is any amino acid;

$X_6$  is any amino acid;

$X_7$  is L, I, V, M, A, F, Y or W;

$X_8$  is C, T or S;

$X_9$  is R, K or H;

20

$X_{10}$  is any amino acid;

$X_{11}$  is any amino acid;

$X_{12}$  is L, I, V, M, A or P;

$X_{13}$  is any amino acid;

$X_{14}$  is any amino acid;

25

$X_{15}$  is any amino acid;

$X_{16}$  is L, I, V, M, A, P, G, C, T or S;

$[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;

30

$X_{17}$  is L, I, V, M, A or P;

$X_{18}$  is any amino acid;



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$X_{19}$  is any amino acid;

$X_{20}$  L, I, V, M, A or P;

$X_{21}$  is P;

$X_{22}$  is L, I, V, M, A, P or G;

5  $X_{23}$  is P or N;

$[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids and wherein the sequence  $X_j$  may comprise the same or different amino acids selected from any amino acid residue;

$X_{24}$  is L, I, V, M, A or P;

10  $X_{25}$  is any amino acid;

$X_{26}$  is any amino acid;

$X_{27}$  is Y or F;

$X_{28}$  is L, I, V, M, A or P;

15 and a protein:molecule interacting region such as but not limited to one or more of an SH2 domain, WD-40 repeats and/or ankyrin repeats N-terminal of the SOCS box.

Another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or a nucleotide sequence capable of  
20 hybridizing thereto under low stringency conditions at 42°C wherein said protein exhibits the following characteristics:

(i) comprises a SOCS box in its C-terminal region having the amino acid sequence:

25  $X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} [X]_n X_{17} X_{18} X_{19} X_{20}$   
 $X_{21} X_{22} X_{23} [X]_n X_{24} X_{25} X_{26} X_{27} X_{28}$

wherein:  $X_1$  is L, I, V, M, A or P;

$X_2$  is any amino acid residue;

30  $X_3$  is P, T or S;

$X_4$  is L, I, V, M, A or P;

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- $X_5$  is any amino acid;  
 $X_6$  is any amino acid;  
 $X_7$  is L, I, V, M, A, F, Y or W;  
 $X_8$  is C, T or S;  
5  $X_9$  is R, K or H;  
 $X_{10}$  is any amino acid;  
 $X_{11}$  is any amino acid;  
 $X_{12}$  is L, I, V, M, A or P;  
 $X_{13}$  is any amino acid;  
10  $X_{14}$  is any amino acid;  
 $X_{15}$  is any amino acid;  
 $X_{16}$  is L, I, V, M, A, P, G, C, T or S;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
and wherein the sequence  $X_i$  may comprise the same or different amino  
15 acids selected from any amino acid residue;  
 $X_{17}$  is L, I, V, M, A or P;  
 $X_{18}$  is any amino acid;  
 $X_{19}$  is any amino acid;  
 $X_{20}$  L, I, V, M, A or P;  
20  $X_{21}$  is P;  
 $X_{22}$  is L, I, V, M, A, P or G;  
 $X_{23}$  is P or N;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
and wherein the sequence  $X_j$  may comprise the same or different amino  
25 acids selected from any amino acid residue;  
 $X_{24}$  is L, I, V, M, A or P;  
 $X_{25}$  is any amino acid;  
 $X_{26}$  is any amino acid;  
 $X_{27}$  is Y or F;  
30  $X_{28}$  is L, I, V, M, A or P; and

(ii) comprises at least one of a SH2 domain, WD-40 repeats and/or ankyrin repeats or other

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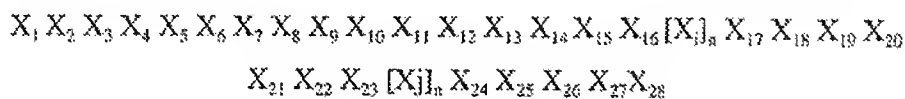
protein:molecule interacting domain in a region N-terminal of the SOCS box.

Preferably, the SOCS molecules modulate signal transduction such as from a cytokine or hormone or other endogenous or exogenous molecule, a microbe or microbial product, an antigen or a parasite.

More preferably, the SOCS molecule modulate cytokine mediated signal transduction.

Still another aspect of the present invention comprises a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a protein or a derivative, homologue, analogue or mimetic thereof or comprises a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein exhibits the following characteristics;

- (i) is capable of modulating signal transduction;
- 15 (ii) comprises a SOCS box in its C-terminal region having the amino acid sequence:



- 20 wherein:  $X_1$  is L, I, V, M, A or P;  
 $X_2$  is any amino acid residue;  
 $X_3$  is P, T or S;  
 $X_4$  is L, I, V, M, A or P;  
 $X_5$  is any amino acid;  
25  $X_6$  is any amino acid;  
 $X_7$  is L, I, V, M, A, F, Y or W;  
 $X_8$  is C, T or S;  
 $X_9$  is R, K or H;  
 $X_{10}$  is any amino acid;  
30  $X_{11}$  is any amino acid;  
 $X_{12}$  is L, I, V, M, A or P;

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$X_{13}$  is any amino acid;

$X_{14}$  is any amino acid;

$X_{15}$  is any amino acid;

$X_{16}$  is L, I, V, M, A, P, G, C, T or S;

5  $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;

$X_{17}$  is L, I, V, M, A or P;

$X_{18}$  is any amino acid;

10  $X_{19}$  is any amino acid;

$X_{20}$  L, I, V, M, A or P;

$X_{21}$  is P;

$X_{22}$  is L, I, V, M, A, P or G;

$X_{23}$  is P or N;

15  $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids and wherein the sequence  $X_j$  may comprise the same or different amino acids selected from any amino acid residue;

$X_{24}$  is L, I, V, M, A or P;

$X_{25}$  is any amino acid;

20  $X_{26}$  is any amino acid;

$X_{27}$  is Y or F;

$X_{28}$  is L, I, V, M, A or P; and

(iii) comprises at least one of a SH2 domain, WD-40 repeats and/or ankyrin repeats or other  
25 protein:molecule interacting domain in a region N-terminal of the SOCS box.

Preferably, the signal transduction is mediated by a cytokine such as one or more of EPO, TPO, G-CSF, GM-CSF, IL-3, IL-2, IL-4, IL-7, IL-13, IL-6, LIF, IL-12, IFN $\alpha$ , TNF $\alpha$ , IL-1 and/or M-CSF.

30

Preferably, the signal transduction is mediated by one or more of Interleukin 6 (IL-6), Leukaemia

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Inhibitory Factor (LIF), Oncostatin M (OSM), Interferon (IFN)- $\alpha$  and/or thrombopoietin.

Preferably, the signal transduction is mediated by IL-6.

5 Particularly preferred nucleic acid molecules comprise nucleotide sequences substantially set forth in SEQ ID NO:3 (mSOCS1), SEQ ID NO:5 (mSOCS2), SEQ ID NO:7 (mSOCS3), SEQ ID NO:9 (hSOCS1), SEQ ID NO:11 (rSOCS1), SEQ ID NO:13 (mSOCS4), SEQ ID NO:15 and SEQ ID NO:16 (hSOCS4), SEQ ID NO:17 (mSOCS5), SEQ ID NO:19 (hSOCS5), SEQ ID NO:20 (mSOCS6), SEQ ID NO:22 and SEQ ID NO:23 (hSOCS6), SEQ ID NO:24  
10 (mSOCS7), SEQ ID NO:26 and SEQ ID NO:27 (hSOCS7), SEQ ID NO:28 (mSOCS8), SEQ ID NO:30 (mSOCS9), SEQ ID NO:31 (hSOCS9), SEQ ID NO:32 (mSOCS10), SEQ ID NO:33 and SEQ ID NO:34 (hSOCS10), SEQ ID NO:35 (hSOCS11), SEQ ID NO:37 (mSOCS12), SEQ ID NO:38 and SEQ ID NO:39 (hSOCS12), SEQ ID NO:40 (mSOCS13), SEQ ID NO:42 (hSOCS13), SEQ ID NO: 43 (mSOCS14), SEQ ID NO:45 (mSOCS15) and SEQ ID NO:47  
15 (hSOCS15) or a nucleotide sequence having at least about 15% similarity to all or a region of any of the listed sequences or a nucleotide acid molecule capable of hybridizing to any one of the listed sequences under low stringency conditions at 42°C.

Another aspect of the present invention relates to a protein or a derivative, homologue, analogue  
20 or mimetic thereof comprising a SOCS box in its C-terminal region.

Yet another aspect of the present invention is directed to a protein or a derivative, homologue, analogue or mimetic thereof comprising a SOCS box in its C-terminal region and a protein:molecule interacting region.  
25

Even yet another aspect of the present invention provides a protein or a derivative, homologue, analogue or mimetic thereof comprising an interacting region located in a region N-terminal of the SOCS box.

30 Preferably, the protein:molecule interacting region is a protein:DNA or a protein:protein binding region.

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Another aspect of the present invention contemplates a protein or a derivative, homologue, analogue or mimetic thereof comprising a SOCS box in its C-terminal region and a SH2 domain, WD-40 repeats or ankyrin repeats N-terminal of the SOCS box.

5 Still yet another aspect of the present invention provides a protein or a derivative, homologue, analogue or mimetic thereof exhibiting the following characteristics:

(i) comprises a SOCS box in its C-terminal region having the amino acid sequence:

10  $X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} [X]_n X_{17} X_{18} X_{19} X_{20}$   
 $X_{21} X_{22} X_{23} [X]_n X_{24} X_{25} X_{26} X_{27} X_{28}$

wherein:  $X_1$  is L, I, V, M, A or P;

$X_2$  is any amino acid residue;

15  $X_3$  is P, T or S;

$X_4$  is L, I, V, M, A or P;

$X_5$  is any amino acid;

$X_6$  is any amino acid;

$X_7$  is L, I, V, M, A, F, Y or W;

20  $X_8$  is C, T or S;

$X_9$  is R, K or H;

$X_{10}$  is any amino acid;

$X_{11}$  is any amino acid;

$X_{12}$  is L, I, V, M, A or P;

25  $X_{13}$  is any amino acid;

$X_{14}$  is any amino acid;

$X_{15}$  is any amino acid;

$X_{16}$  is L, I, V, M, A, P, G, C, T or S;

$[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids

30 and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;

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- 5  $X_{17}$  is L, I, V, M, A or P;  
 $X_{18}$  is any amino acid;  
 $X_{19}$  is any amino acid;  
 $X_{20}$  L, I, V, M, A or P;  
 $X_{21}$  is P;  
 $X_{22}$  is L, I, V, M, A, P or G;  
 $X_{23}$  is P or N;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
 and wherein the sequence  $X_j$  may comprise the same or different amino  
 10 acids selected from any amino acid residue;  
 $X_{24}$  is L, I, V, M, A or P;  
 $X_{25}$  is any amino acid;  
 $X_{26}$  is any amino acid;  
 $X_{27}$  is Y or F;  
 15  $X_{28}$  is L, I, V, M, A or P; and

(ii) comprises at least one of a SH2 domain, WD-40 repeats and/or ankyrin repeats or other protein:molecule interacting domain in a region N-terminal of the SOCS box.

- 20 Preferably, the proteins modulate signal transduction such as cytokine-mediated signal transduction.

Preferred cytokines are EPO, TPO, G-CSF, GM-CSF, IL-3, IL-2, IL-4, IL-7, IL-13, IL-6, LIF, IL-12, IFN $\gamma$ , TNF $\alpha$ , IL-1 and/or M-CSF.

25

A particularly preferred cytokine is IL-6.

Even yet another aspect of the present invention provides a protein or derivative, homologue, analogue or mimetic thereof exhibiting the following characteristics:

- 30 (i) is capable of modulating signal transduction such as cytokine-mediated signal transduction;

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- (ii) comprises a SOCS box in its C-terminal region having the amino acid sequence:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} [X_i]_n X_{17} X_{18} X_{19} X_{20} \\ X_{21} X_{22} X_{23} [X_j]_m X_{24} X_{25} X_{26} X_{27} X_{28}$$

5

wherein:  $X_1$  is L, I, V, M, A or P;

$X_2$  is any amino acid residue;

$X_3$  is P, T or S;

$X_4$  is L, I, V, M, A or P;

10

$X_5$  is any amino acid;

$X_6$  is any amino acid;

$X_7$  is L, I, V, M, A, F, Y or W;

$X_8$  is C, T or S;

$X_9$  is R, K or H;

15

$X_{10}$  is any amino acid;

$X_{11}$  is any amino acid;

$X_{12}$  is L, I, V, M, A or P;

$X_{13}$  is any amino acid;

$X_{14}$  is any amino acid;

20

$X_{15}$  is any amino acid;

$X_{16}$  is L, I, V, M, A, P, G, C, T or S;

$[X_i]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;

25

$X_{17}$  is L, I, V, M, A or P;

$X_{18}$  is any amino acid;

$X_{19}$  is any amino acid;

$X_{20}$  L, I, V, M, A or P;

$X_{21}$  is P;

30

$X_{22}$  is L, I, V, M, A, P or G;

$X_{23}$  is P or N;



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$[X_i]_n$  is a sequence of  $n$  amino acids wherein  $n$  is from 1 to 50 amino acids and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;

$X_{24}$  is L, I, V, M, A or P;

5  $X_{25}$  is any amino acid;

$X_{26}$  is any amino acid;

$X_{27}$  is Y or F;

$X_{28}$  is L, I, V, M, A or P; and

- 10 (iii) comprises at least one of a SH2 domain, WD-40 repeats and/or ankyrin repeats or other protein-molecule interacting domain in a region N-terminal of the SOCS box.

Particularly preferred SOCS proteins comprise an amino acid sequence substantially as set forth in SEQ ID NO:4 (mSOCS1), SEQ ID NO:6 (mSOCS2), SEQ ID NO:8 (mSOCS3), SEQ ID  
15 NO:10 (hSOCS1), SEQ ID NO:12 (rSOCS1), SEQ ID NO:14 (mSOCS4), SEQ ID NO:18 (mSOCS5), SEQ ID NO:21 (mSOCS6), SEQ ID NO:25 (mSOCS7), SEQ ID NO:29 (mSOCS8), SEQ ID NO:36 (hSOCS11), SEQ ID NO:41 (mSOCS13), SEQ ID NO:44 (mSOCS14), SEQ ID NO:46 (mSOCS15) and SEQ ID NO:48 (hSOCS15) or an amino acid sequence having at least 15% similarity to all or a region of any one of the listed sequences.

20

Another aspect of the present invention contemplates a method of modulating levels of a SOCS protein in a cell said method comprising contacting a cell containing a SOCS gene with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time and under conditions sufficient to modulate levels of said SOCS protein.

25

A related aspect of the present invention provides a method of modulating signal transduction in a cell containing a SOCS gene comprising contacting said cell with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time sufficient to modulate signal transduction.

30

Yet a further related aspect of the present invention is directed to a method of influencing

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interaction between cells wherein at least one cell carries a SOCS gene, said method comprising contacting the cell carrying the SOCS gene with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time sufficient to modulate signal transduction.

- 5 In accordance with the present invention,  $n$  in  $[X_i]_n$  and  $[X_j]_n$  may, in addition from being 1-50, be from 1-30, 1-20, 1-10 and 1-5.

A summary of the SEQ ID NOs referred to in the subject specification is given in Table 1.

10

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**TABLE 1**  
**SUMMARY OF SEQUENCE IDENTITY NUMBERS**

	<b>SEQUENCE</b>	<b>SEQ ID NO.</b>
5	PCR Primer	1
	PCR Primer	2
	Mouse SOCS1 (nucleotide)	3
	Mouse SOCS1 (amino acid)	4
10	Mouse SOCS2 (nucleotide)	5
	Mouse SOCS2 (amino acid)	6
	Mouse SOCS3 (nucleotide)	7
	Mouse SOCS3 (amino acid)	8
	Human SOCS1 (nucleotide)	9
15	Human SOCS1 (amino acid)	10
	Rat SOCS1 (nucleotide)	11
	Rat SOCS1 (amino acid)	12
	nucleotide sequence of murine SOCS4	13
	amino acid sequence of murine SOCS4	14
20	nucleotide sequence of SOCS4 cDNA human contig 4.1	15
	nucleotide sequence of SOCS4 cDNA human contig 4.2	16
	nucleotide sequence of murine SOCS5	17
	amino acid sequence of murine SOCS5	18
	nucleotide sequence of human SOCS5	19
25	nucleotide sequence of murine SOCS6	20
	amino acid of murine SOCS6	21
	nucleotide sequence of human SOCS6 contig h6.1	22
	nucleotide sequence of human SOCS6 contig h6.2	23
	nucleotide sequence of murine SOCS7	24

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	amino acid sequence of murine SOCS7	25
	nucleotide sequence of human SOCS7 contig h7.1	26
	nucleotide sequence of human SOCS7 contig 17.2	27
	nucleotide sequence of murine SOCS8	28
5	amino acid sequence of murine SOCS 8	29
	nucleotide sequence of murine SOCS9	30
	nucleotide sequence of human SOCS9	31
	nucleotide sequence of murine SOCS10	32
	nucleotide sequence of human SOCS10 contig h10.1	33
10	nucleotide sequence of human SOCS10 contig h10.2	34
	nucleotide sequence of human SOCS11	35
	amino acid sequence of human SOCS11	36
	nucleotide sequence of mouse SOCS12	37
	nucleotide sequence of human SOCS12 contig h12.1	38
15	nucleotide sequence of human SOCS12 contig h12.2	39
	nucleotide sequence of murine SOCS13	40
	amino acid sequence of murine SOCS13	41
	nucleotide sequence of human SOCS13 cDNA contig h13.1	42
	nucleotide sequence of murine SOCS14 cDNA	43
20	amino acid sequence of murine SOCS14	44
	nucleotide sequence of murine SOCS15 cDNA	45
	amino acid sequence of murine SOCS15	46
	nucleotide sequence of human SOCS15	47
	amino acid sequence of human SOCS15	48
25		

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Single and three letter abbreviations are used to denote amino acid residues and these are summarized in Table 2.

TABLE 2

5	Amino Acid	Three-letter Abbreviation	One-letter Symbol
	Alanine	Ala	A
10	Arginine	Arg	R
	Asparagine	Asn	N
	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
15	Glutamic acid	Glu	E
	Glycine	Gly	G
	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
20	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
25	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
	Any residue	Xaa	X
30			

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**BRIEF DESCRIPTION OF THE DRAWINGS**

In some of the Figures, abbreviations are used to denote SOCS proteins with certain binding motifs. SOCS proteins which contain WD-40 repeats are referred to as WSB1-WSB4. SOCS  
5 proteins with ankyrin repeats are referred to as ASB1-ASB3.

**Figure 1** is a diagrammatic representation showing generation of an IL-6-unresponsive M1 clone by retroviral infection. The RUFneo retrovirus, showing the position of landmark restriction endonuclease cleavage sites, the 4A2 cDNA insert and the position of PCR primer sequences.

10

**Figure 2** is a photographic representation of Southern and Northern analysis. (Left and Middle Panels) Southern blot analysis of genomic DNA from clone 4A2 and a control infected M1 clone. DNA was digested with BamHI, to reveal the number of retroviruses carried by each clone, and Sac I, to estimate the size of the retroviral cDNA insert. Left panel; probed with neo. Right  
15 panel; probed with the Xho I-digested 4A2 PCR product. (Right Panel) Northern blot analysis of total RNA from clone 4A2 and a control infected M1 clone, probed with the Xho I-digested 4A2 PCR product. The two bands represent unspliced and spliced retroviral transcripts, resulting from splice donor and acceptor sites in the retroviral genome.

20 **Figure 3** is a representation of the nucleotide sequence and structure of the SOCS1 gene. A. The genomic context of SOCS1 in relation to the protamine gene cluster on murine chromosome 16. The accession number of this locus is MPMRGMNS (direct submission; G. Schlueter, 1995) for the mouse and BTPRMTNP2 for the rat (direct submission; G. Schlueter, 1996). B. The nucleotide sequence of the SOCS1 cDNA and deduced amino acid sequence. Conventional one  
25 letter abbreviations are used for the amino acid sequence and the asterisk indicates the stop codon. The polyadenylation signal sequence is underlined. The coding region is shown in uppercase and the untranslated region is shown in lower case.

**Figure 4** is a graphical representation of cell differentiation in the presence of cytokines. Semi-  
30 solid agar cultures of parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1), were used and the percentage of colonies which differentiated in response

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to a titration of 1 mg/ml IL-6 (●), 100 ng/ml LIF (◇), 1 mg/ml OSM (□), 100 ng/ml IFN- $\gamma$  (▲), 500 ng/ml TPO (●), or  $3 \times 10^{-6}$  M dexamethasone (✱) determined.

- Figure 5** is a photographic representation of cytopins of liquid cultures of parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) cultured for 4 days in the presence of 10 ng/ml IL-6 or saline. Unlike parental M1 cells, morphological features consistent with macrophage differentiation are not observed in M1 cells constitutively expressing SOCS1 (4A2 and M1.mpl.SOCS1) when cultured in IL-6.
- Figure 6** is a photographic representation showing inhibition of phosphorylation of signalling molecules by SOCS1. Parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) were incubated in the absence (-) or presence (+) of 10 ng/ml of IL-6 for 4 minutes at 37°C. Cells were then lysed and extracts were either immunoprecipitated using anti-mouse gp130 antibody prior to SDS-PAGE (two upper panels) or were electrophoresed directly (two lower panels). Gels were blotted and the filters were then probed with anti-phosphotyrosine (upper panel), anti-gp130 antibody (second top panel), anti-phospho-STAT3 (second bottom panel) or anti-STAT3 (lower panel). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents.
- Figure 7** is a representation of protein extracts prepared from (A) M1 cells or M1 cells expressing SOCS1 (4A2) and (B) M1.mpl cells or M1.mpl.SOCS1 cells incubated for 10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN- $\gamma$ . The binding reactions contained 4-6  $\mu$ g protein (constant within a given experiment), 5 ng  $^{32}$ P-labelled m67 oligonucleotide encoding the high affinity SIF (c-sis- inducible factor) binding site, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled m67 oligonucleotide, or antibodies specific for either STAT1 or STAT3.

**Figure 8** is a photographic representation of Northern hybridisation. Mice were injected intravenously with 2  $\mu$ g and after various periods of time, the livers were removed and polyA+

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mRNA was purified. M1 cells were stimulated for various lengths of time with 500 ng/ml of IL-6, after which polyA+ mRNA was isolated. mRNA was fractionated by electrophoresis and immobilized on nylon filters. Northern blots were prehybridized, hybridized with random-primed <sup>32</sup>P-labelled SOCS1 or GAPDH DNA fragments, washed and exposed to film overnight.

5

**Figure 9** is a representation of a comparison of the amino acid sequences of SOCS1, SOCS2, SOCS3 and CIS. Alignment of the predicted amino acid sequence of mouse (mm), human (hs) and rat (rr) SOCS1, SOCS2, SOCS3 and CIS. Those residues shaded are conserved in three or four mouse SOCS family members. The SH2 domain is boxed in solid lines, while the SOCS box  
10 is bounded by double lines.

**Figure 10** is a photographic representation showing the phenotype of IL-6 unresponsive M1 cell clone, 4A2. Colonies of parental M1 cells (left panel) and clone 4A2 (right panel) cultured in semi-solid agar for 7 days in saline or 100 ng/ml IL-6.

15

**Figure 11** is a photographic representation showing expression of mRNA for SOCS family members *in vitro* and *in vivo*.

(A) Northern analysis of mRNA from a range of mouse organs showing constitutive expression of SOCS family members in a limited number of tissues.

20 (B) Northern analysis of mRNA from liver and M1 cells showing induction of expression of SOCS family members following exposure to IL-6.

(C) Reverse transcriptase PCR analysis of mRNA from bone marrow showing induction of expression of SOCS family members by a range of cytokines.

25 **Figure 12** is a photographic representation showing SOCS1 suppresses the phosphorylation and activation of gp130 and STAT-3.

(A) Western blots of extracts from parental M1 cells (M1 and M1.mpl) and M1 cells expressing SOCS1 (4A2 and M1.mpl.SOCS1) stimulated with (+) or without (-) 100 ng/ml IL-6. Top: Extracts immunoprecipitated with anti-gp130 ( $\alpha$ gp130) and immunoblotted with anti-phosphotyrosine ( $\alpha$ PY-STAT3), or for STAT3 ( $\alpha$ STAT3) to demonstrate equal loading of  
30 protein. The molecular weights of the bands are shown on the right.



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(B) EMSA of M1.mpl and M1.mpl.SOCS1 cells stimulated with (+) and without (-) 100 ng/ml IL-6 or 100 ng/ml IFN $\gamma$ . The DNA-binding complexes SIF A, B, and C are indicated at the left.

5 **Figure 13** is a representation of a comparison of the amino acid sequence of the SOCS proteins  
 (A) Schematic representation of structures of SOCS proteins including proteins which contain  
 WD-40 repeats (WSB) and ankyrin repeats (ASB). (B) Alignment of N-terminal regions of  
 SOCS proteins. (C) Alignment of the SH2 domains of CIS, SOCS1, 2, 3, 5, 9, 11 and 14. (D)  
 Alignment of the WD-40 repeats of SOCS4, SOCS6, SOCS13 and SOCS15. (E) Alignment of  
 10 the ankyrin repeats of SOCS7 and SOCS10. (F) Alignment of the regions between SH2, WD-40  
 and ankyrin repeats and the SOCS box. (G) Alignment of the SOCS box. In each case the  
 conventional one letter abbreviations for amino acids are used, with X denoting residues of  
 uncertain identity and OOO denoting the beginning and the end of contigs. Amino acid  
 sequence obtained from conceptual translation of nucleic acid sequence derived from isolated  
 15 cDNAs is shown in upper case while amino acid sequence obtained by conceptual translation of  
 ESTs is shown in lower case and is approximate only. Conserved residues, defined as (LIVMA),  
 (FYW), (DE), (QN), (C, S, T), (KRH), (PG) are shaded in the SH2 domain, WD-40 repeats,  
 ankyrin repeats and the SOCS box. For the alignment of SH2 domains, WD-40 repeats and  
 ankyrin repeats a consensus sequence is shown above. In each case this has been derived from  
 20 examination of a large and diverse set of domains (Neer *et al*, 1994; Bork, 1993).

**Figures 14(A) and (B)** are photographic representations showing analysis of mRNA expression  
 of mouse SOCS1 and SOCS5 and SOCS containing a WD-40 repeat (WSB2) and ankyrin  
 repeats (ASB1).

25

**Figure 15** is a representation showing the nucleotide sequence of the mouse SOCS4 cDNA. The  
 nucleotides encoding the mature coding region from the predicted ATG "start" codon to the stop  
 codon is shown in upper case, while the predicted 5' and 3' untranslated regions are shown in  
 lower case. The relationship of mouse cDNA sequence to mouse and human EST contigs is  
 30 illustrated in Figure 17.

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**Figure 16** is a representation showing the predicted amino acid sequence of the mouse SOCS4 protein, derived from the nucleotide sequence in **Figure 15**. The SOCS box, which also shown in **Figure 13**, is underlined.

5 **Figure 18** is a representation showing the nucleotide sequence of human SOCS4 cDNA contigs h4.1 and h4.2, derived from analysis of ESTs listed in Table 4.1. The relationship of these contigs to the mouse cDNA sequence is illustrated in **Figure 17**.

**Figure 19** is a diagrammatic representation showing the relationship of mouse SOCS5 genomic  
10 (57-2) and cDNA (5-3-2) clones to contigs derived from analysis of mouse ESTs (Table 5.1) and human cDNA clone (5-94-2) and ESTs (Table 5.2). The nucleotide sequence of the mouse SOCS5 contig is shown in **Figure 20**, with the sequence of human SOCS5 contig (h5.1) being shown in **Figure 21**. The deduced amino acid sequence of mouse SOCS5 is shown in **Figure 20B**. The structure of the protein is shown schematically, with the SH2 domain indicated by  
15 ( ) and the SOCS box by ( ). The putative 5' and 3' translated regions are shown by the thin solid line.

**Figure 20A** is a representation showing the nucleotide sequence of the mouse SOCS5 derived from analysis of genomic and cDNA clones. The nucleotides encoding the mature coding region  
20 from the predicted ATG "start" codon to the stop codon is shown in upper case, while the predicted 5' and 3' untranslated regions are shown in lower case. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in **Figure 19**.

**Figure 20B** is a representation of the predicted amino acid sequence of mouse SOCS5 protein,  
25 derived from the nucleotide sequence in **Figure 20A**. The SOCS box, which also shown in **Figure 13** is underlined.

**Figure 21** is a representation showing the nucleotide sequence of human SOCS5 cDNA contig h5.1, derived from analysis of cDNA clone 5-94-2 and the ESTs listed in Table 5.2. The  
30 relationship of these contigs to the mouse cDNA sequence is illustrated in **Figure 19**.

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**Figure 22** is a diagrammatic representation showing the relationship of mouse SOCS6 cDNA clones (6-1A, 6-2A, 6-5B, 6-4N, 6-18, 6-29, 6-3N and 6-5N) to contigs derived from analysis of mouse ESTs (Table 6.1) and human ESTs (Table 6.2). The nucleotide sequence of the mouse SOCS-6 contig is shown in Figure 23, with the sequence of human SOCS6 contigs (h6.1 and h6.2) being shown in Figure 24. The deduced amino acid sequence of mouse SOCS6 is shown in Figure 23B. The structure of the protein is shown schematically, while the WD-40 repeats indicated by ( ) and the SOCS box by ( ). The putative 5' and 3' untranslated regions are shown by the thin solid line.

10 **Figure 23A** is a representation showing the nucleotide sequence of the mouse SOCS6 derived from analysis of cDNA clone 64-10A-11. The nucleotides encoding the part of the predicted coding region, ending in the stop codon are shown in upper case, while the predicted 3' untranslated regions are shown in lower case. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in Figure 22.

15

**Figure 23B** is a representation showing the predicted amino acid sequence of mouse SOCS6 protein, derived from the nucleotide sequence in Figure 23A. The SOCS box, which also shown in Figure 13 is underlined.

20 **Figure 24** is a representation showing the nucleotide sequence of human SOCS6 cDNA contig h6.1, derived from analysis of cDNA clone 5-94-2 and the ESTs listed in Table 6.2. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 22

**Figure 25** is a diagrammatic representation showing the relationship of mouse SOCS7 cDNA clone (74-10A-11) to contigs derived from analysis of mouse ESTs (Table 7.1) and human ESTs (Table 7.2). The nucleotide sequence of the mouse SOCS7 contig is shown in Figure 26 with the sequence of human SOCS7 contigs (h7.1 and h7.2) being shown in Figure 27. The deduced amino acid sequence of mouse SOCS7 is shown in Figure 26B. The structure of the protein is shown schematically, with the ankyrin repeats indicated by ( ) and the SOCS box by ( ). The putative 5' and 3' untranslated regions are shown by the thin solid line in the mouse and by the wavy line in h7.2. Based on analysis of clones isolated to date and ESTs the 3' untranslated

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regions of mSOCS7 and hSOCS7 share little similarity.

**Figure 26A** is a representation showing the nucleotide sequence of the mouse SOCS7 derived from analysis of cDNA clone 74-10A-11. The nucleotides encoding the part of the predicted coding region, ending in the stop codon are shown in upper case, while the predicted 3' untranslated regions are shown in lower case. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in Figure 25.

**Figure 26B** is a representation showing the predicted amino acid sequence of mouse SOCS7 protein, derived from the nucleotide sequence in Figure 26A. The SOCS box, which also shown in Figure 13 is underlined.

**Figure 27** is a representation showing the nucleotide sequence of human SOCS7 cDNA contig h7.1 and h7.2 derived from analysis of the ESTs listed in Table 7.2. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 25.

**Figure 28** is a diagrammatic representation of the relationship of sequence derived from analysis of mouse SOCS8 ESTs (Table 8.1 and Figure 29A) to the predicted protein structure of mouse SOCS8. The deduced partial amino acid sequence of mouse SOCS8 is shown in Figure 29B. The structure of the protein is shown schematically with the SOCS box highlighted ( ). The predicted 3' untranslated region is shown by the thin line.

**Figure 29A** is a representation showing the partial nucleotide sequence of mouse SOCS8 cDNA (contig 8.1) derived from analysis of ESTs. The nucleotides encoding the part of the predicted coding region, ending in the STOP codon are shown in upper case, while the predicted 3' untranslated regions are shown in lower case.

**Figure 29B** is a representation showing the partial predicted amino acid sequence of the mouse SOCS8 protein, derived from the nucleotide sequence in Figure 29A. The SOCS box, which also shown in Figure 13 is underlined.

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Figure 30 is a diagrammatic representation showing the relationship of mouse SOCS9 ESTs (Table 9.1) and human SOCS9 ESTs (Table 9.2). The nucleotide sequence of the mouse SOCS9 contig (m9.1) is shown in Figure 31, with the sequence of human SOCS9 contig (h9.1) being shown in Figure 32. The deduced amino acid sequence of human SOCS9 is shown schematically, with the SH2 domain indicated by ( ) and the SOCS box by ( ). The putative 3' untranslated region is shown by the thin solid line.

Figure 31 is a representation showing the partial nucleotide sequence of mouse SOCS9 cDNA (contig m9.1), derived from analysis of the ESTs listed in Table 9.1. The relationship of these 10 contigs to the mouse cDNA sequence is illustrated in Figure 30.

Figure 32 is a representation showing the partial nucleotide sequence of human SOCS9 cDNA (contig h9.1), derived from analysis of the ESTs listed in Table 9.2. Although it is clear that contig h9.1 encodes a protein with an SH2 domain and a SOCS box, the quality of the sequence 15 is not high enough to derive a single unambiguous open reading frame. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 30.

Figure 33 is a representation showing the relationship of mouse SOCS10 cDNA clones (10-9, 10-12, 10-23 and 10-24) to contigs derived from analysis of mouse ESTs (Table 10.1) and 20 human ESTs (Table 10.2). The nucleotide sequence of the mouse SOCS10 contig is shown in Figure 10.2, with the sequence of human SOCS10 contigs (h10.1 and h10.2) being shown in Figure 35. The predicted structure of the protein is shown schematically, with the ankyrin repeats indicated by ( ) and the SOCS box by ( ). The putative 3' untranslated regions is shown by the thin line solid line in the mouse and by the wavy line in h10.2. Based on analysis of clones 25 isolated to date and ESTs the 3' untranslated regions of mSOCS-10 and hSOCS-10 share little similarity.

Figure 34 is a representation showing the nucleotide sequence of the mouse SOCS10 derived from analysis of cDNA clone 10-9, 10-12, 10-23 and 10-24. The nucleotides encoding the part 30 of the predicted coding region, ending in the stop codon are shown in upper case, while the predicted 3' untranslated regions are shown in lower case. Although it is clear that contig m10.1

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encodes a protein with a series of ankyrin repeats and a SOCS box, the quality of the sequence is not high enough to derive a single unambiguous open reading frame. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in Figure 33.

5 **Figure 35** is a representation showing the nucleotide sequence of human SOCS10 cDNA contig h10.2 and h10.2 derived from analysis of the ESTs listed in Table 10.2. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 33.

**Figure 36A** is a representation showing the partial nucleotide sequence of the human SOCS11 cDNA derived from analysis of ESTs listed in Table 11.1. The nucleotides encoding the mature  
10 coding region from the predicted ATG "start" codon to the stop codon is shown in upper case, while the predicted 5' and 3' untranslated regions are shown in lower case. The relationship of the partial cDNA sequence, derived from ESTs, to the predicted protein is shown in Figure 37.

**Figure 36B** is a representation showing the partial predicted amino acid sequence of human  
15 SOCS11 protein, derived from the nucleotide sequence in Figure 36A. The SOCS box, which also shown in Figure 13, is underlined.

**Figure 37** is a diagrammatic representation showing the relationship of sequence derived from analysis of human SOCS-11 ESTs (Table 11.1 and Figure 36A) to the predicted protein structure  
20 of human SOCS11. The deduced partial amino acid sequence of human SOCS11 is shown in Figure 36B. The structure of the protein is shown schematically with the SH2 domain shown by ( ) and the SOCS box highlighted by ( ). The predicted 3' untranslated region is shown by the thin line.

25 **Figure 38** is a diagrammatic representation showing the relationship of mouse SOCS12 cDNA clones (12-1) to contigs derived from analysis of mouse ESTs (Table 12.1) and human ESTs (Table 12.2). The nucleotide sequence of the mouse SOCS12 contig is shown in Figure 12.2, with the sequence of human SOCS12 contigs (h12.1 and h12.2) being shown in Figure 40. The deduced partial amino acid sequence of mouse SOCS12 is shown in Figure 39. The structure  
30 of the protein is shown schematically, with the ankyrin repeats indicated by ( ) and the SOCS box by ( ). The putative 3' untranslated region is shown by the thin line solid line in the mouse and

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by the wavy line in h12.2. Based on analysis of clones isolated to date and ESTs the 3' untranslated regions of mSOCS12 and hSOCS12 share little similarity.

**Figure 39** is a representation showing the nucleotide sequence of the mouse SOCS12 derived from analysis of cDNA clone 12-1 and the ESTs listed in Table 12.1. The nucleotides encoding the part of the predicted coding region, including the stop codon are shown in upper case, while the predicted 3' untranslated region is shown in lower case. By homology with human SOCS12 it is clear that contig m12.1 encodes a protein with a series of ankyrin repeats and a SOCS box, the quality of the sequence is not high enough to derive a single unambiguous open reading frame. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in Figure 38.

**Figure 40** is a representation showing the nucleotide sequence of human SOCS12 cDNA contig h12.1 and h12.2 derived from analysis of the ESTs listed in Table 12.2. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 38.

**Figure 41** is a diagrammatic representation showing the relationship of contig m13.1 derived from analysis of mouse SOCS13 cDNA clones (62-1, 62-6-7, 62-14) and mouse ESTs (Table 13.1) to contig h13.1 derived from analysis of human ESTs (Table 13.2). The nucleotide sequence of the mouse SOCS13 contig is shown in Figure 42, with the sequence of human SOCS13 contig (h13.1) being shown in Figure 43. The deduced amino acid sequence of mouse SOCS13 is shown in Figure 42B. The structure of the protein is shown schematically, with the WD-40 repeats highlighted by ( ) and the SOCS box highlighted by ( ). The 3' untranslated region is shown by the thin line solid line.

**Figure 42A** is a representation showing the nucleotide sequence of the mouse SOCS13 derived from analysis of cDNA clones 62-1, 62-6-7 and 62-14. The nucleotides encoding part of the predicted coding region, ending in the stop codon are shown in upper case, while those encoding the predicted 3' untranslated regions are shown in lower case. The relationship of mouse cDNA sequence to mouse and human EST contigs is illustrated in Figure 41.

- 30 -

**Figure 42B** is a representation showing the predicted amino acid sequence of mouse SOCS13 protein, derived from the nucleotide sequence in Figure 42A. The SOCS box, which also shown in Figure 13 is underlined.

5 **Figure 43** is a representation showing the nucleotide sequence of human SOCS13 cDNA contig h13.1 derived from analysis of the ESTs listed in Table 13.2. The relationship of these contigs to the mouse cDNA sequence is illustrated in Figure 41.

**Figure 44** is a diagrammatic representation showing the relationship of a partial mouse SOCS14  
10 cDNA clone (14-1) to contigs derived from analysis of mouse ESTs (Table 14.1). The nucleotide sequence of the mouse SOCS14 contig is shown in Figure 45. The deduced partial amino acid sequence of mouse SOCS14 is shown in Figure 45B. The structure of the protein is shown schematically, with the SH3 domain indicated by ( ) and the SOCS box by ( ). The putative 3' untranslated region is shown by the thin line.

15

**Figure 45A** is a representation showing the nucleotide sequence of the mouse SOCS14 derived from analysis of genomic and cDNA clones. The nucleotides encoding the mature coding region from the predicted ATG "start" codon to the stop codon is shown in upper case, while the predicted 5' and 3' untranslated regions are shown in lower case. The relationship of mouse  
20 cDNA sequence to mouse and human EST contigs is illustrated in Figure 44.

**Figure 45B** is a representation showing the predicted amino acid sequence of mouse SOCS14 protein, derived from the nucleotide sequence in Figure 45B. The SOCS box, which also shown in Figure 13 is underlined.

25

**Figure 46** is a diagrammatic representation showing the relationship of contig m15.1 derived from analysis of mouse BAC and mouse ESTs (Table 15.1) to contig h15.1 derived from analysis of the human BAC and human ESTs (Table 15.2). The nucleotide sequence of the mouse SOCS15 contig is shown in Figure 47, with the sequence of human SOCS15 contig (h15.1)  
30 being shown in Figure 47. The deduced amino acid sequence of mouse SOCS15 is shown in Figure 47B. The structure of the protein is shown schematically, with the WD-40 repeats



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highlighted by ( ) and the SOCS box highlighted by ( ). The 5' and 3' untranslated region are shown by the thin line solid line. The introns which interrupt the coding region are shown by ^.

**Figure 47A** is a representation showing the nucleotide sequence covering the mouse SOCS15 gene derived from analysis the mouse BAC listed in Table 15.1. The nucleotides encoding the predicted coding region, beginning with the ATG and ending in the stop codon are shown in upper case, while those encoding the predicted 5' untranslated region, the introns and the 3' untranslated region are shown in lower case. The relationship of mouse BAC to mouse and human ESTs contigs is illustrated in Figure 46.

10

**Figure 47B** is a representation showing the predicted amino acid sequence of mouse SOCS15 protein, derived from the nucleotide sequence in Figure 47A. The SOCS box, which also shown in Figure 13 is underlined.

**Figure 48A** is a representation showing the nucleotide sequence covering the human SOCS15 gene derived from analysis the human BAC listed in Table 15.2. The nucleotides encoding the predicted coding region, beginning with the ATG and ending in the stop codon are shown in upper case, while those encoding the predicted 5' untranslated region, the introns and the 3' untranslated region are shown in lower case. The relationship of the human BAC to mouse and human ESTs contigs is illustrated in Figure 46.

20

**Figure 48B** is a representation showing the predicted amino acid sequence of human SOCS15 protein, derived from the nucleotide sequence in Figure 48A. The SOCS box, which also shown in Figure 13 is underlined.

25

**Figure 49** is a photographic representation showing SOCS1 inhibition of JAK2 kinase activity. (A) Upper panel. Cos M6 cells were transiently transfected with either Flag-tagged mJAK2 and mSOCS-1 DNA (SOCS1) or Flag-mJAK2 DNA alone (-), lysed, JAK2 proteins immunoprecipitated using anti-JAK2 antibody and subjected to an *in vitro* kinase assay. Lower panel. A portion of the JAK2 immunoprecipitates were Western blotted with anti-JAK2 antibody. (B) Upper panel. Cos M6 cells were transiently transfected with Flag- mJAK2 and

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Flag- mSOCS-1 DNA or Flag-mJAK2 DNA alone, lysed, JAK2 proteins immunoprecipitated using anti-JAK2 (UBI) and separated by SDS/PAGE gel. Immunoprecipitates were then analysed by Western blot with anti-phosphotyrosine antibody. Lower panel; JAK2 expression. Cos cell lysates were separated by SDS/PAGE gel and analysed by Western blot with anti-FLAG  
5 antibody (M2).

**Figure 50** is a photographic representation showing interaction between JAK2 and SOCS protein. (A) Cos M6 cells were transiently transfected with Flag-tagged mJAK2 and various Flag-tagged SOCS DNAs (SOCS-1;S1, SOCS-2;S2, SOCS-3;S3, CIS) or Flag-mJAK2 alone,  
10 lysed, JAK2 proteins immunoprecipitated using anti-JAK2 (UBI) and separated by SDS/PAGE. Immunoprecipitates were then analysed by Western blot with anti-FLAG antibody (M2). (B) Cos cell lysates described in (A) were separated by SDS/PAGE and expression levels of the various proteins were determined by Western blot with anti-FLAG antibody (M2). (C) JAK2 tyrosine phosphorylation. Cos cell lysates described in (A) were separated by SDS/PAGE and  
15 proteins analysed by Western blot with anti-phosphotyrosine antibody.

**Figure 51** is a diagrammatic representation of p $\beta$ galpAloxneo.

**Figure 52** is a diagrammatic representation of p $\beta$ galpAloxneoTK.

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**Figure 53** is a diagrammatic representation of SOCS1 knockout construct.

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**DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

The present invention provides a new family of modulators of signal transduction. As the initial members of this family suppressed cytokine signalling, the family is referred to as the "suppressors of cytokine signalling" family of "SOCS". The SOCS family is defined by the presence of a C-terminal domain referred to as a "SOCS box". Different classes of SOCS molecules are defined by a motif generally but not exclusively located N-terminal to the SOCS box and which is involved by protein:molecule interaction such as protein:DNA or protein:protein interaction. Particularly preferred motifs are selected from an SH2 domain, WD-40 repeats and ankyrin repeats.

WD-40 repeats were originally recognised in the  $\beta$ -subunit of G-proteins. WD-40 repeats appear to form a  $\beta$ -propeller-like structure and may be involved in protein-protein interactions. Ankyrin repeats were originally recognised in the cytoskeletal protein ankyrin.

Members of the SOCS family may be identified by any number of means. For example, SOCS1 to SOCS3 were identified by their ability to suppress cytokine-mediated signal transduction and, hence, were identified based on activity. SOCS4 to SOCS15 were identified as nucleotide sequences exhibiting similarity at the level of the SOCS box.

The SOCS box is a conserved motif located in the C-terminal region of the SOCS molecule. In accordance with the present invention, the amino acid sequence of the SOCS box is:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} [X_{17}]_R X_{17} X_{18} X_{19} X_{20} \\ X_{21} X_{22} X_{23} [X_{24}]_R X_{24} X_{25} X_{26} X_{27} X_{28}$$

wherein:  $X_1$  is L, I, V, M, A or P;  
 $X_2$  is any amino acid residue;  
 $X_3$  is P, T or S;  
 $X_4$  is L, I, V, M, A or P;  
 $X_5$  is any amino acid;

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- $X_6$  is any amino acid;  
 $X_7$  is L, I, V, M, A, F, Y or W;  
 $X_8$  is C, T or S;  
 $X_9$  is R, K or H;  
5  $X_{10}$  is any amino acid;  
 $X_{11}$  is any amino acid;  
 $X_{12}$  is L, I, V, M, A or P;  
 $X_{13}$  is any amino acid;  
 $X_{14}$  is any amino acid;  
10  $X_{15}$  is any amino acid;  
 $X_{16}$  is L, I, V, M, A, P, G, C, T or S;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
and wherein the sequence  $X_i$  may comprise the same or different amino  
acids selected from any amino acid residue;  
15  $X_{17}$  is L, I, V, M, A or P;  
 $X_{18}$  is any amino acid;  
 $X_{19}$  is any amino acid;  
 $X_{20}$  L, I, V, M, A or P;  
 $X_{21}$  is P;  
20  $X_{22}$  is L, I, V, M, A, P or G;  
 $X_{23}$  is P or N;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
and wherein the sequence  $X_i$  may comprise the same or different amino  
acids selected from any amino acid residue;  
25  $X_{24}$  is L, I, V, M, A or P;  
 $X_{25}$  is any amino acid;  
 $X_{26}$  is any amino acid;  
 $X_{27}$  is Y or F; and  
 $X_{28}$  is L, I, V, M, A or P.

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As stated above and in accordance with the present invention, SOCS proteins are divided into

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separate classes based on the presence of a protein:molecule interacting region such as but not limited to an SH2 domain, WD-40 repeats and ankyrin repeats located N-terminal of the SOCS box. The latter three domains are protein:protein interacting domains.

- 5 Examples of SH2 containing SOCS proteins include SOCS1, SOCS2, SOCS3, SOCS5, SOCS9, SOCS11 and SOCS14. Examples of SOCS containing WD-40 repeats include SOCS4, SOCS6 and SOCS15. Examples of SOCS containing ankyrin repeats include SOCS7, SOCS10 and SOCS12.
- 10 The present invention provides *inter alia* nucleic acid molecules encoding SOCS proteins, purified naturally occurring SOCS proteins as well as recombinant forms of SOCS proteins and methods of modulating signal transduction by modulating activity of SOCS proteins or expression of SOCS genes. Preferably, signal transduction is mediated by a cytokine, examples of which include EPO, TPO, G-CSF, GM-CSF, IL-3, IL-2, IL-4, IL-7, IL-13, IL-6, LIF, IL-12,
- 15 IFN $\gamma$ , TNF $\alpha$ , IL-1 and/or M-CSF. Particularly preferred cytokines include IL-6, LIF, OSM, IFN- $\gamma$  and/or thrombopoietin.

Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a

20 protein or a derivative, homologue, analogue or mimetic thereof or comprises a nucleotide sequence capable of hybridizing thereto under low stringency conditions at 42°C wherein said protein comprises a SOCS box in its C-terminal region and optionally a protein:molecule interacting domain N-terminal of the SOCS box.

- 25 Preferably, the protein:molecule interacting domain is a protein:DNA or protein:protein interacting domain. Most preferably, the protein:molecule interacting domain is one of an SH2 domain, WD-40 repeats and/or ankyrin repeats.

As stated above, preferably the subject SOCS modulate cytokine-mediated signal transduction.

30 The present invention extends, however, to SOCS molecules modulating other effector-mediated signal transduction such as mediated by other endogenous or exogenous molecules, antigens,

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microbes and microbial products, viruses or components thereof, ions, hormones and parasites. Endogenous molecules in this context are molecules produced within the cell carrying the SOCS molecule. Exogenous molecules are produced by other cells or are introduced to the body.

- 5 Preferably, the nucleic acid molecule or SOCS protein is in isolated or purified form. The terms "isolated" and "purified" mean that a molecule has undergone at least one purification step away from other material.

Preferably, the nucleic acid molecule is in isolated form and is DNA such as cDNA or genomic  
10 DNA. The DNA may encode the same amino acid sequence as the naturally occurring SOCS or the SOCS may contain one or more amino acid substitutions, deletions and/or additions. The nucleotide sequence may correspond to the genomic coding sequence (including exons and introns) or to the nucleotide sequence in cDNA from mRNA transcribed from the genomic gene or it may carry one or more nucleotide substitutions, deletions and/or additions thereto.

15

In a preferred embodiment, the nucleic acid molecule comprises a sequence of nucleotide encoding or complementary to a sequence encoding a SOCS protein or a derivative, homologue, analogue or mimetic thereof wherein the amino acid sequence of said SOCS protein is selected from SEQ ID NO:4 (mSOCS1), SEQ ID NO:6 (mSOCS2), SEQ ID NO:8 (mSOCS3), SEQ ID  
20 NO:10 (hSOCS1), SEQ ID NO:12 (rSOCS1), SEQ ID NO:14 (mSOCS4), SEQ ID NO:18 (mSOCS5), SEQ ID NO:21 (mSOCS6), SEQ ID NO:25 (mSOCS27), SEQ ID NO:29 (mSOCS8), SEQ ID NO:36 (hSOCS11), SEQ ID NO:41 (mSOCS13), SEQ ID NO:44 (mSOCS14), SEQ ID NO:46 (mSOCS15) and SEQ ID NO:48 (mSOCS15) or encodes an amino acid sequence with a single or multiple amino acid substitution, deletion and/or addition to the  
25 listed sequences or is a nucleotide sequence capable of hybridizing to the nucleic acid molecule under low stringency conditions at 42°C.

In an even more preferred embodiment, the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a  
30 SOCS protein or a derivative, homologue, analogue or mimetic thereof wherein the nucleotide sequence is selected from a nucleotide sequence substantially set forth in SEQ ID NO:3

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(mSOCS1), SEQ ID NO:5 (mSOCS2), SEQ ID NO:7 (mSOCS3), SEQ ID NO:9 (hSOCS11),  
 SEQ ID NO:11 (rSOCS1), SEQ ID NO:13 (mSOCS4), SEQ ID NO:15 and SEQ ID NO:16  
 (hSOCS4), SEQ ID NO:17 (mSOCS5), SEQ ID NO:19 (hSOCS5), SEQ ID NO:20 (mSOCS6),  
 SEQ ID NO:22 and SEQ ID NO:23 (hSOCS6), SEQ ID NO:24 (mSOCS7), SEQ ID NO:26 and  
 5 SEQ ID NO:27 (hSOCS7), SEQ ID NO:28 (mSOCS8), SEQ ID NO:30 (mSOCS9), SEQ ID  
 NO:31 (hSOCS9), SEQ ID NO:32 (mSOCS10), SEQ ID NO:33 and SEQ ID NO:34  
 (hSOCS10), SEQ ID NO:35 (hSOCS11), SEQ ID NO:37 (mSOCS12), SEQ ID NO:38 and  
 SEQ ID NO:39 (hSOCS12), SEQ ID NO:40 (mSOCS13), SEQ ID NO:42 (hSOCS13), SEQ  
 ID NO:43 (mSOCS14), SEQ ID NO:45 (mSOCS15) and SEQ ID NO:47 (hSOCS15) or a  
 10 nucleotide sequence having at least about 15% similarity to all or a region of any of the listed  
 sequences or a nucleic acid molecule capable of hybridizing to any of the listed sequences under  
 low stringency conditions at 42°C.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1%  
 15 v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for  
 hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative  
 stringency conditions may be applied where necessary, such as medium stringency, which  
 includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and  
 from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M  
 20 to at least about 0.9M salt for washing conditions, or high stringency, which includes and  
 encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least  
 about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least  
 about 0.15M salt for washing conditions.

25 In another embodiment, the present invention is directed to a SOCS protein or a derivative,  
 homologue, analogue or mimetic thereof wherein said SOCS protein is identified as follows:

human SOCS4 characterised by EST81149, EST180909, EST182619, ya99H09,  
 ye70co4, yh53c09, yh77g11, yh87h05, yi45h07, yj04e06, yq12h06, yq56a06, yq60e02,  
 30 yq92g03, yq97h06, yr90f01, yt69c03, yv30a08, yv55f07, yv57h09, yv87h02, yv98e11,  
 yw68d10, yw82a03, yx08a07, yx72h06, yx76b09, yy37h08, yy66b02, za81f08, zb18f07,

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zc06e08, zd14g06, zd51h12, zd52b09, ze25g11, ze69f02, zf54f03, zh96e07, zv66h12, zs83a08 and zs83g08;

mouse SOCS-4 characterised by mc65f04, mf42e06, mp10c10, mr81g09, and mt19h12;

5

human SOCS-5 characterised by EST15B103, EST15B105, EST27530 and zf50f01;

mouse SOCS-5 characterised by mc55a01, mh98f09, my26h12 and ve24e06;

10

human SOCS-6 characterised by yf61e08, yf93a09, yg05f12, yg41f04, yg45c02, yh11f10, yh13b05, zc35a12, ze02h08, zl09a03, zl69e10, zn39d08 and zo39e06;

15

mouse SOCS-6 characterised by mc04c05, md48a03, mf31d03, mh26b07, mh78e11, mh88h09, mh94h07, mi27h04 and mj29c05, mp66g04, mw75g03, va53b05, vb34h02, vc55d07, vc59e05, vc67d03, vc68d10, vc97h01, vc99c08, vd07h03, vd08c01, vd09b12, vd19b02, vd29a04 and vd46d06;

20

human SOCS-7 characterised by STS WI30171, EST00939, EST12913, yc29b05, yp49f10, zt10f03 and zx73g04;

mouse SOCS-7 characterised by mj39a01 and vi52h07;

mouse SOCS-8 characterised by mj6e09 and vj27a029;

25

human SOCS-9 characterised by CSRL-82f2-u, EST114054, yy06b07, yy06g06, zr40c09, zr72b01, yx92c08, yx93b08 and hfe0662;

mouse SOCS-9 characterised by me65d05;

30

human SOCS-10 characterised by aa48h10, zp35h01, zp97h12, zq08h01, zr34g05, EST73000 and HSDHEI005;



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mouse SOCS-10 characterised by mb14d12, mb40f06, mg89b11, mq89e12, mp03g12 and vh53c11;

human SOCS-11 characterised by zt24h06 and zr43b02;

5

human SOCS-13 characterised by EST59161;

mouse SOCS-13 characterised by ma39a09, mc60c05, mi78g05, mk10c11, mo48g12, mp94a01, vb57c07 and vh07c11; and

10

human SOCS-14 characterised by mi75c03, vd29h11 and vd53g07;

or a derivative or homologue of the above ESTs characterised by a nucleic acid molecule being capable of hybridizing to any of the listed ESTs under low stringency conditions at 42°C.

15

In another embodiment, the nucleotide sequence encodes the following amino acid sequence:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12} X_{13} X_{14} X_{15} X_{16} [X_i]_n X_{17} X_{18} X_{19} X_{20} \\ X_{21} X_{22} X_{23} [X_j]_n X_{24} X_{25} X_{26} X_{27} X_{28}$$

20

wherein:  $X_1$  is L, I, V, M, A or P;  
 $X_2$  is any amino acid residue;  
 $X_3$  is P, T or S;  
 $X_4$  is L, I, V, M, A or P;  
 $X_5$  is any amino acid;  
 $X_6$  is any amino acid;  
 $X_7$  is L, I, V, M, A, F, Y or W;  
 $X_8$  is C, T or S;  
 $X_9$  is R, K or H;  
 $X_{10}$  is any amino acid;  
 $X_{11}$  is any amino acid;

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- $X_{12}$  is L, I, V, M, A or P;  
 $X_{13}$  is any amino acid;  
 $X_{14}$  is any amino acid;  
 $X_{15}$  is any amino acid;  
 5  $X_{16}$  is L, I, V, M, A, P, G, C, T or S;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
 and wherein the sequence  $X_j$  may comprise the same or different amino  
 acids selected from any amino acid residue;  
 $X_{17}$  is L, I, V, M, A or P;  
 10  $X_{18}$  is any amino acid;  
 $X_{19}$  is any amino acid;  
 $X_{20}$  L, I, V, M, A or P;  
 $X_{21}$  is P;  
 $X_{22}$  is L, I, V, M, A, P or G;  
 15  $X_{23}$  is P or N;  
 $[X]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
 and wherein the sequence  $X_j$  may comprise the same or different amino  
 acids selected from any amino acid residue;  
 $X_{24}$  is L, I, V, M, A or P;  
 20  $X_{25}$  is any amino acid;  
 $X_{26}$  is any amino acid;  
 $X_{27}$  is Y or F; and  
 $X_{28}$  is L, I, V, M, A or P.

- 25 The above sequence comparisons are preferably to the whole molecule but may also be to part  
 thereof. Preferably, the comparisons are made to a contiguous series of at least about 21  
 nucleotides or at least about 5 amino acids. More preferably, the comparisons are made against  
 at least about 21 contiguous nucleotides or at least 7 contiguous amino acids. Comparisons may  
 also only be made to the SOCS box region or a region encompassing the protein:molecule  
 30 interacting region such as the SH2 domain WD-40 repeats and/or ankyrin repeats.

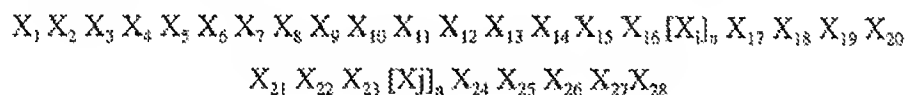
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Still another embodiment of the present invention contemplates an isolated polypeptide or a derivative, homologue, analogue or mimetic thereof comprising a SOCS box in its C-terminal region.

- 5 Preferably the polypeptide further comprises a protein:molecule interacting domain such as a protein:DNA or protein:protein interacting domain. Preferably, this domain is located N-terminal of the SOCS box. It is particularly preferred for the protein:molecule interacting domain to be at least one of an SH2 domain, WD-40 repeats and/or ankyrin repeats.
- 10 Preferably, the signal transduction is mediated by a cytokine selected from EPO, TPO, G-CSF, GM-CSF, IL-3, IL-2, IL-4, IL-7, IL-13, IL-6, LIF, IL-12, IFN $\gamma$ , TNF $\alpha$ , IL-1 and/or M-CSF. Preferred cytokines are IL-6, LIF, OSM, IFN- $\gamma$  or thrombopoietin.

More preferably, the protein comprises a SOCS box having the amino acid sequence:

15



wherein:  $X_1$  is L, I, V, M, A or P;

20

$X_2$  is any amino acid residue;

$X_3$  is P, T or S;

$X_4$  is L, I, V, M, A or P;

$X_5$  is any amino acid;

$X_6$  is any amino acid;

25

$X_7$  is L, I, V, M, A, F, Y or W;

$X_8$  is C, T or S;

$X_9$  is R, K or H;

$X_{10}$  is any amino acid;

$X_{11}$  is any amino acid;

30

$X_{12}$  is L, I, V, M, A or P;

$X_{13}$  is any amino acid;

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- $X_{14}$  is any amino acid;  
 $X_{15}$  is any amino acid;  
 $X_{16}$  is L, I, V, M, A, P, G, C, T or S;  
 $[X_i]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
 5 and wherein the sequence  $X_i$  may comprise the same or different amino acids selected from any amino acid residue;  
 $X_{17}$  is L, I, V, M, A or P;  
 $X_{18}$  is any amino acid;  
 $X_{19}$  is any amino acid;  
 10  $X_{20}$  L, I, V, M, A or P;  
 $X_{21}$  is P;  
 $X_{22}$  is L, I, V, M, A, P or G;  
 $X_{23}$  is P or N;  
 $[X_j]_n$  is a sequence of n amino acids wherein n is from 1 to 50 amino acids  
 15 and wherein the sequence  $X_j$  may comprise the same or different amino acids selected from any amino acid residue;  
 $X_{24}$  is L, I, V, M, A or P;  
 $X_{25}$  is any amino acid;  
 $X_{26}$  is any amino acid;  
 20  $X_{27}$  is Y or F; and  
 $X_{28}$  is L, I, V, M, A or P.

Still another embodiment provides an isolated polypeptide or a derivative, homologue, analogue or mimetic thereof comprising a sequence of amino acids substantially as set forth in SEQ ID  
 25 NO:4 (mSOCS1), SEQ ID NO:6 (mSOCS2), SEQ ID NO:8 (mSOCS3), SEQ ID NO:10 (hSOCS1), SEQ ID NO:12 (rSOCS1), SEQ ID NO:14 (mSOCS4), SEQ ID NO:18 (mSOCS5), SEQ ID NO:21 (mSOCS6), SEQ ID NO:25 (mSOCS7), SEQ ID NO:29 (mSOCS8), SEQ ID NO:36 (hSOCS11), SEQ ID NO:41 (mSOCS13), SEQ ID NO:44 (mSOCS14), SEQ ID NO:46 (mSOCS15) and SEQ ID NO:48 (hSOCS15) or an amino acid sequence having at least 15%  
 30 similarity to all or a part of the listed sequences.

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Preferred nucleotide percentage similarities include at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or above such as 93%, 95%, 98% or 99%.

- 5 Preferred amino acid similarities include at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 97% or 98% or above.

As stated above, similarity may be measured against an entire molecule or a region comprising  
10 at least 21 nucleotides or at least 7 amino acids. Preferably, similarity is measured in a conserved region such as SH2 domain, WD-40 repeats, ankyrin repeats or other protein:molecule interacting domains or a SOCS box.

The term "similarity" includes exact identity between sequences or, where the sequence differs,  
15 different amino acids are related to each other at the structural, functional, biochemical and/or conformational levels.

The nucleic acid molecule may be isolated from any animal such as humans, primates, livestock animals (e.g. horses, cows, sheep, donkeys, pigs), laboratory test animals (e.g. mice, rats, rabbits,  
20 hamsters, guinea pigs), companion animals (e.g. dogs, cats) or captive wild animals (e.g. deer, foxes, kangaroos).

The terms "derivatives" or its singular form "derivative" whether in relation to a nucleic acid molecule or a protein includes parts, mutants, fragments and analogues as well as hybrid or  
25 fusion molecules and glycosylation variants. Particularly useful derivatives comprise single or multiple amino acid substitutions, deletions and/or additions to the SOCS amino acid sequence.

Preferably, the derivatives have functional activity or alternatively act as antagonists or agonists. The present invention further extends to homologues of SOCS which include the functionally or  
30 structurally related molecule from different animal species. The present invention also encompasses analogues and mimetics. Mimetics include a class of molecule generally but not

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necessarily having a non-amino acid structure and which functionally are capable of acting in an analogous manner to the protein for which it is a mimic, in this case, a SOCS. Mimetics may comprise a carbohydrate, aromatic ring, lipid or other complex chemical structure or may also be proteinaceous in composition. Mimetics as well as agonists and antagonists contemplated  
5 herein are conveniently located through systematic searching of environments, such as coral, marine and freshwater river beds, flora and microorganisms. This is sometimes referred to as natural product screening. Alternatively, libraries of synthetic chemical compounds may be screened for potentially useful molecules.

10 As stated above, the present invention contemplates agonists and antagonists of the SOCS. One example of an antagonist is an antisense oligonucleotide sequence. Useful oligonucleotides are those which have a nucleotide sequence complementary to at least a portion of the protein-coding or "sense" sequence of the nucleotide sequence. These anti-sense nucleotides can be used to effect the specific inhibition of gene expression. The antisense approach can cause  
15 inhibition of gene expression apparently by forming an anti-parallel duplex by complementary base pairing between the antisense construct and the targeted mRNA, presumably resulting in hybridisation arrest of translation. Ribozymes and co-suppression molecules may also be used. Antisense and other nucleic acid molecules may first need to be chemically modified to permit penetration of cell membranes and/or to increase their serum half life or otherwise make them  
20 more stable for *in vivo* administration. Antibodies may also act as either antagonists or agonists although are more useful in diagnostic applications or in the purification of SOCS proteins. Antagonists and agonists may also be identified following natural product screening or screening of libraries of chemical compounds or may be derivatives or analogues of the SOCS molecules.

25

Accordingly, the present invention extends to analogues of the SOCS proteins of the present invention. Analogues may be used, for example, in the treatment or prophylaxis of cytokine mediated dysfunction such as autoimmunity, immune suppression or hyperactive immunity or other condition including but not limited to dysfunctions in the haemopoietic, endocrine, hepatic  
30 and neural systems. Dysfunctions mediated by other signal transducing elements such as hormones or endogenous or exogenous molecules, antigens, microbes and microbial products,

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viruses or components thereof, ions, hormones and parasites are also contemplated by the present invention.

Analogues of the proteins contemplated herein include, but are not limited to, modification to  
5 side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include  
10 modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with  $\text{NaBH}_4$ ; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-  
15 phosphate followed by reduction with  $\text{NaBH}_4$ .

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

20 The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides  
25 with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

30 Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides.

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Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with  
5 iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine,  
10 sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acid, contemplated herein is shown in Table 3.



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TABLE 3

Non-conventional amino acid	Code	Non-conventional amino acid	Code
5			
$\alpha$ -aminobutyric acid	Abu	L-N-methylalanine	Nmala
$\alpha$ -amino- $\alpha$ -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
10 carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
15 cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
20 D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
25 D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
30 D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle

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	D-tryptophan	Dtrp	L-norvaline	Nva
	D-tyrosine	Dtyr	$\alpha$ -methyl-aminoisobutyrate	Maib
	D-valine	Dval	$\alpha$ -methyl- $\gamma$ -aminobutyrate	Mgabv
	D- $\alpha$ -methylalanine	Dmala	$\alpha$ -methylcyclohexylalanine	Mchexa
5	D- $\alpha$ -methylarginine	Dmarg	$\alpha$ -methylcyclopentylalanine	Mcpvn
	D- $\alpha$ -methyiasparagine	Dmasn	$\alpha$ -methyl- $\alpha$ -naphthylalanine	Manap
	D- $\alpha$ -methyiaspartate	Dmasp	$\alpha$ -methylpenicillamine	Mpen
	D- $\alpha$ -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nghu
	D- $\alpha$ -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
10	D- $\alpha$ -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
	D- $\alpha$ -methylisoleucine	Dmile	N-amino- $\alpha$ -methylbutyrate	Nmaabu
	D- $\alpha$ -methylleucine	Dmleu	$\alpha$ -naphthylalanine	Anap
	D- $\alpha$ -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- $\alpha$ -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Nglu
15	D- $\alpha$ -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
	D- $\alpha$ -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nghu
	D- $\alpha$ -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- $\alpha$ -methylserine	Dmser	N-cyclobutylglycine	Nebut
	D- $\alpha$ -methylthreonine	Dmthr	N-cycloheptyl gly cine	Nchep
20	D- $\alpha$ -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
	D- $\alpha$ -methyltyrosine	Dmtv	N-cyclodecylglycine	Nedec
	D- $\alpha$ -methylvaline	Dmval	N-cylcododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
25	D-N-methyiasparagine	Dnmasn	N-cycloundecylglycine	Ncund
	D-N-methyiaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl)glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
30	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nscr
	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl)glycine	Nhis

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	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- $\gamma$ -aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
5	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaiib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
10	D-N-methyltyrosine	Dnmtyr	N-methyl-naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	$\gamma$ -aminobutyric acid	Gabu	N-( <i>p</i> -hydroxyphenyl)glycine	Nhtyr
	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
15	L-homophenylalanine	Hphe	L- $\alpha$ -methylalanine	Mala
	L- $\alpha$ -methylarginine	Marg	L- $\alpha$ -methyiasparagine	Masn
	L- $\alpha$ -methylaspartate	Masp	L- $\alpha$ -methyl- <i>t</i> -butylglycine	Mtbug
	L- $\alpha$ -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- $\alpha$ -methylglutamine	Mgln	L- $\alpha$ -methylglutamate	Mglu
20	L- $\alpha$ -methylhistidine	Mhis	L- $\alpha$ -methylhomophenylalanine	Mhphe
	L- $\alpha$ -methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L- $\alpha$ -methylleucine	Mleu	L- $\alpha$ -methyllysine	Mlys
	L- $\alpha$ -methylmethionine	Mmet	L- $\alpha$ -methylnorleucine	Mnle
	L- $\alpha$ -methylnorvaline	Mnva	L- $\alpha$ -methylornithine	Morn
25	L- $\alpha$ -methylphenylalanine	Mphe	L- $\alpha$ -methylproline	Mpro
	L- $\alpha$ -methylserine	Mser	L- $\alpha$ -methylthreonine	Mthr
	L- $\alpha$ -methyltryptophan	Mtrp	L- $\alpha$ -methyltyrosine	Mtyr
	L- $\alpha$ -methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe

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N-(N-(2,2-diphenylethyl)	Nubhm	N-(N-(3,3-diphenylpropyl)	Nabbe
carbamylmethyl)glycine		carbamylmethyl)glycine	
1-carboxy-1-(2,2-diphenyl-	Nmbc		
ethylamino)cyclopropane			

5

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having  $(CH_2)_n$  spacer groups with  $n=1$  to  $n=6$ , glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually

10 contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of  $C_\alpha$  and  $N_\epsilon$ -methylamino acids, introduction of double bonds between  $C_\alpha$  and  $C_\beta$  atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming

15 an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

These types of modifications may be important to stabilise the cytokines if administered to an individual or for use as a diagnostic reagent.

20

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

25

Another embodiment of the present invention contemplates a method for modulating expression of a SOCS protein in a mammal, said method comprising contacting a gene encoding a SOCS or a factor/element involved in controlling expression of the SOCS gene with an effective amount of a modulator of SOCS expression for a time and under conditions sufficient

30 to up-regulate or down-regulate or otherwise modulate expression of SOCS. An example of a modulator is a cytokine such as IL-6 or other transcription regulators of SOCS expression.

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Expression includes transcription or translation or both.

Another aspect of the present invention contemplates a method of modulating activity of SOCS in a human, said method comprising administering to said mammal a modulating effective  
5 amount of a molecule for a time and under conditions sufficient to increase or decrease SOCS activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of SOCS or a chemical analogue or truncation mutant of SOCS.

A further aspect of the present invention provides a method of inducing synthesis of a SOCS  
10 or transcription/translation of a SOCS comprising contacting a cell containing a SOCS gene with an effective amount of a cytokine capable of inducing said SOCS for a time and under conditions sufficient for said SOCS to be produced. For example, SOCS1 may be induced by IL-6.

15 Still a further aspect of the present invention contemplates a method of modulating levels of a SOCS protein in a cell said method comprising contacting a cell containing a SOCS gene with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time and under conditions sufficient to modulate levels of said SOCS protein.

20 Yet a further aspect of the present invention contemplates a method of modulating signal transduction in a cell containing a SOCS gene comprising contacting said cell with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time sufficient to modulate signal transduction.

25 Even yet a further aspect of the present invention contemplates a method of influencing interaction between cells wherein at least one cell carries a SOCS gene, said method comprising contacting the cell carrying the SOCS gene with an effective amount of a modulator of SOCS gene expression or SOCS protein activity for a time sufficient to modulate signal transduction.

30 As stated above, of the present invention contemplates a range of mimetics or small molecules capable of acting as agonists or antagonists of the SOCS. Such molecules may be obtained

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from natural product screening such as from coral, soil, plants or the ocean or antarctic environments. Alternatively, peptide, polypeptide or protein libraries or chemical libraries may be readily screened. For example, M1 cells expressing a SOCS do not undergo differentiation in the presence of IL-6. This system can be used to screen molecules which permit  
5 differentiation in the presence of IL-6 and a SOCS. A range of test cells may be prepared to screen for antagonists and agonists for a range of cytokines. Such molecules are preferably small molecules and may be of amino acid origin or of chemical origin. SOCS molecules interacting with signalling proteins (eg. JAKS) provide molecular screens to detect molecules which interfere or promote this interaction. Once such screening protocol involves natural  
10 product screening.

Accordingly, the present invention contemplates a pharmaceutical composition comprising SOCS or a derivative thereof or a modulator of SOCS expression or SOCS activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to  
15 as the "active ingredients". These and other aspects of the present invention apply to any SOCS molecules such as but not limited to SOCS1 to SOCS15.

The pharmaceutical forms containing active ingredients suitable for injectable use include sterile aqueous solutions (where water soluble) sterile powders for the extemporaneous preparation  
20 of sterile injectable solutions. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for  
25 example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.  
30 Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

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Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the  
5 freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft  
10 shell gelatin capsule, or it may be compressed into tablets. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may  
15 conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1  $\mu$ g and 2000 mg of active compound.

20

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter. A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or  
25 saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound,  
30 sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any

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dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

- 5 The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and  
10 the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

- 15 It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for  
20 the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

25

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5  $\mu$ g to about 2000 mg. Expressed in proportions, the  
30 active compound is generally present in from about 0.5  $\mu$ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are



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determined by reference to the usual dose and manner of administration of the said ingredients. The effective amount may also be conveniently expressed in terms of an amount per kg of body weight. For example, from about 0.01 ng to about 10,000 mg/kg body weight may be administered.

5

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating SOCS expression or SOCS activity. The vector may, for example, be a viral vector. In this regard, a range of gene therapies are contemplated by the present invention including  
10 isolating certain cells, genetically manipulating and returning the cell to the same subject or to a genetically related or similar subject.

Still another aspect of the present invention is directed to antibodies to SOCS and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from  
15 naturally occurring antibodies to SOCS or may be specifically raised to SOCS or derivatives thereof. In the case of the latter, SOCS or its derivatives may first need to be associated with a carrier molecule. The antibodies and/or recombinant SOCS or its derivatives of the present invention are particularly useful as therapeutic or diagnostic agents.

20 For example, SOCS and its derivatives can be used to screen for naturally occurring antibodies to SOCS. These may occur, for example in some autoimmune diseases. Alternatively, specific antibodies can be used to screen for SOCS. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of SOCS levels may be important for diagnosis of certain cancers or a predisposition to cancers or monitoring cytokine  
25 mediated cellular responsiveness or for monitoring certain therapeutic protocols.

Antibodies to SOCS of the present invention may be monoclonal or polyclonal. Alternatively, fragments of antibodies may be used such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic  
30 antibody" is considered herein to include fragments and hybrids of antibodies. The antibodies of this aspect of the present invention are particularly useful for immunotherapy and may also

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be used as a diagnostic tool for assessing apoptosis or monitoring the program of a therapeutic regimen.

For example, specific antibodies can be used to screen for SOCS proteins. The latter would be  
5 important, for example, as a means for screening for levels of SOCS in a cell extract or other biological fluid or purifying SOCS made by recombinant means from culture supernatant fluid. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

10 It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of SOCS.

15

Both polyclonal and monoclonal antibodies are obtainable by immunization with the enzyme or protein and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of SOCS, or  
20 antigenic parts thereof, collecting serum from the animal, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

25 The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

30

Another aspect of the present invention contemplates a method for detecting SOCS in a

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biological sample from a subject said method comprising contacting said biological sample with an antibody specific for SOCS or its derivatives or homologues for a time and under conditions sufficient for an antibody-SOCS complex to form and then detecting said complex.

5 The presence of SOCS may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. These, of course, include both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in the traditional competitive binding assays. These assays also include direct binding of a labelled  
10 antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay,  
15 an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-  
20 antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are  
25 added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain SOCS including cell extract, tissue biopsy or possibly serum, saliva, mucosal secretions, lymph, tissue fluid and respiratory fluid. The sample is, therefore, generally a biological sample comprising biological fluid but also  
30 extends to fermentation fluid and supernatant fluid such as from a cell culture.

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In the typical forward sandwich assay, a first antibody having specificity for the SOCS or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports  
5 may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40  
10 minutes or overnight if more convenient) and under suitable conditions (e.g. room temperature to 37°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

15

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody.  
20 Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its  
25 chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

30 In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a

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wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding  
5 enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then  
10 added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

15

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a  
20 characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are  
25 particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

The present invention also contemplates genetic assays such as involving PCR analysis to detect SOCS gene or its derivatives. Alternative methods or methods used in conjunction include  
30 direct nucleotide sequencing or mutation scanning such as single stranded conformation polymorphisms analysis (SSCP) as specific oligonucleotide hybridisation, as methods such as

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direct protein truncation tests.

Since cytokines are involved in transcription of some SOCS molecules, the detection of SOCS provides surrogate markers for cytokines or cytokine activity. This may be useful in assessing  
5 subjects with a range of conditions such as those with autoimmune diseases, for example, rheumatoid arthritis, diabetes and stiff man syndrome amongst others.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic  
10 acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and  
15 expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

20 Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and a mammalian and more particularly a human SOCS gene portion, which SOCS gene portion is capable of encoding a SOCS polypeptide or a functional or immunologically interactive derivative thereof.

25 Preferably, the SOCS gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said SOCS gene portion in an appropriate cell.

In addition, the SOCS gene portion of the genetic construct may comprise all or part of the  
30 gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

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The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

The present invention also extends to any or all derivatives of SOCS including mutants, part,  
5 fragments, portions, homologues and analogues or their encoding genetic sequence including single or multiple nucleotide or amino acid substitutions, additions and/or deletions to the naturally occurring nucleotide or amino acid sequence. The present invention also extends to mimetics and agonists and antagonists of SOCS.

10 The SOCS and its genetic sequence of the present invention will be useful in the generation of a range of therapeutic and diagnostic reagents and will be especially useful in the detection of a cytokine involved in a particular cellular response or a receptor for that cytokine. For example, cells expressing SOCS gene such as M1 cells expressing the SOCS1 gene, will no longer be responsive to a particular cytokine such as, in the case of SOCS1, IL-6. Clearly, the  
15 present invention further contemplates cells such as M1 cells expressing any SOCS gene such as from SOCS1 to SOCS15. Furthermore, the present invention provides the use of molecules that regulate or potentiate the ability of therapeutic cytokines. For example, molecules which block some SOCS activity, may act to potential therapeutic cytokine activity (eg. G-CSF).

20 Soluble SOCS polypeptides are also contemplated to be particularly useful in the treatment of disease, injury or abnormality involving cytokine mediated cellular responsiveness such as hyperimmunity, immunosuppression, allergies, hypertension and the like.

A further aspect of the present invention contemplates the use of SOCS or its functional  
25 derivatives in the manufacture of a medicament for the treatment of conditions involving cytokine mediated cellular responsiveness.

The present invention further contemplates transgenic mammalian cells expressing a SOCS gene. Such cells are useful indicator cell lines for assaying for suppression of cytokine function.  
30 One example is M1 cells expressing a SOCS gene. Such cell lines may be useful for screening for cytokines or screening molecules such as naturally occurring molecules from plants, coral,

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microorganisms or bio-organically active soil or water capable of acting as cytokine antagonists or agonists.

The present invention further contemplates hybrids between different SOCS from the same or  
5 different animal species. For example, a hybrid may be formed between all or a functional part of mouse SOCS1 and human SOCS1. Alternatively, the hybrid may be between all or part of mouse SOCS1 and mouse SOCS2. All such hybrids are contemplated herein and are particularly useful in developing pleiotropic molecules.

- 10 The present invention further contemplates a range of genetic based diagnostic assays screening for individuals with defective SOCS genes. Such mutations may result in cell types not being responsive to a particular cytokine or resulting in over responsiveness leading to a range of conditions. The SOCS genetic sequence can be readily verified using a range of PCR or other techniques to determine whether a mutation is resident in the gene. Appropriate gene therapy  
15 or other interventionist therapy may then be adopted.

The present invention is further described by the following non-limiting Examples.



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Examples 1-16 relate to SOCS1, SOCS2 and SOCS3 which were identified on the basis of activity. Examples 17-24 relate to various aspects of SOCS4 to SOCS15 which were cloned initially on the basis of sequence similarity. Examples 25-36 relate to specific aspects of SOCS4 to SOCS15, respectively.

5

**EXAMPLE 1****CELL CULTURE AND CYTOKINES**

The M1 cell line was derived from a spontaneously arising leukaemia in SL mice [Ichikawa, 1969]. Parental M1 cells used in this study have been in passage at the Walter and Eliza Hall Institute for Medical Research, Melbourne, Victoria, Australia, for approximately 10 years. M1  
10 cells were maintained by weekly passage in Dulbecco's modified Eagle's medium (DME) containing 10% (v/v) foetal bovine serum (FCS). Recombinant cytokines are generally available from commercial sources or were prepared by published methods. Recombinant murine LIF was produced in *Escherichia coli* and purified, as previously described [Gearing, 1989]. Purified human oncostatin M was purchased from PeproTech Inc (Rocky Hill, NJ,  
15 USA), and purified mouse IFN- $\gamma$  was obtained from Genzyme Diagnostics (Cambridge, MA, USA). Recombinant murine thrombopoietin was produced as a FLAGTM-tagged fusion protein in CHO cells and then purified.

20

**EXAMPLE 2****AGAR COLONY ASSAYS**

In order to assay the differentiation of M1 cells in response to cytokines, 300 cells were cultured in 35 mm Petri dishes containing 1 ml of DME supplemented with 20%(v/v) fetal calf serum (FCS), 0.3%(w/v) agar and 0.1 ml of serial dilutions of IL-6, LIF, OSM, IFN- $\gamma$ , tpo or dexamethasone (Sigma Chemical Company, St Louis, MI). After 7 days culture at 37°C in a  
25 fully humidified atmosphere, containing 10% (v/v) CO<sub>2</sub> in air, colonies of M1 cells were counted and classified as differentiated if they were composed of dispersed cells or had a corona of dispersed cells around a tightly packed centre.

30

**EXAMPLE 3****GENERATION OF RETROVIRAL LIBRARY**

A cDNA expression library was constructed from the factor-dependent haemopoietic cell line

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FDC-P1, essentially as described [Rayner, 1994]. Briefly, cDNA was cloned into the retroviral vector pRUFneo and then transfected into an amphotrophic packaging cell line (PA317). Transiently generated virus was harvested from the cell supernatant at 48 hr posttransfection, and used to infect Y2 ecotropic packaging cells, to generate a high titre virus-producing cell line.

#### EXAMPLE 4

##### RETROVIRAL INFECTION OF M1 CELLS

Pools of  $10^6$  infected  $\Psi$ 2 cells were irradiated (3000 rad) and cocultivated with  $10^6$  M1 cells in DME supplemented with 10%(v/v) FCS and 4  $\mu$ g/ml Polybrene, for 2 days at 37°C. To select for IL-6-unresponsive clones, retrovirally-infected M1 cells were washed once in DME, and cultured at approximately  $2 \times 10^4$  cells/ml in 1 ml agar cultures containing 400  $\mu$ g/ml geneticin (GibcoBRL, Grand Island, NY) and 100 ng/ml IL-6. The efficiency of infection of M1 cells was 1-2%, as estimated by agar plating the infected cells in the presence of geneticin only.

#### EXAMPLE 5

##### PCR

Genomic DNA from retrovirally-infected M1 cells was digested with Sac I and 1  $\mu$ g of phenol/chloroform extracted DNA was then amplified by polymerase chain reaction (PCR). Primers used for amplification of cDNA inserts from the integrated retrovirus were GAG3 (5' CACGCCGCCCCACGTGAAGGC 3' [SEQ ID NO:1]), which corresponds to the vector gag sequence approximately 30 bp 5' of the multiple cloning site, and HSVTK (5' TTCGCCAATGACAAGACGCT 3' [SEQ ID NO:2]), which corresponds to the pMC1neo sequence approximately 200 bp 3' of the multiple cloning site. The PCR entailed an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 2 min, and extension at 72°C for 3 min, followed by a final 10 min extension. PCR products were gel purified and then ligated into the pGEM-T plasmid (Promega, Madison, WI), and sequenced using an ABI PRISM Dye Terminator Cycle Sequencing Kit and a Model 373 Automated DNA Sequencer (Applied Biosystems Inc., Foster City, CA).

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**EXAMPLE 6****CLONING OF cDNAs**

Independent cDNA clones encoding mouse SOCS1 were isolated from a murine thymus cDNA library essentially as described (Hilton *et al*, 1994). The nucleotide and predicted amino acid sequences of mouse SOCS1 cDNA were compared to databases using the BLASTN and TFASTA algorithms (Pearson and Lipman, 1988; Pearson, 1990; Altschul *et al*, 1990). Oligonucleotides were designed from the ESTs encoding human SOCS1 and mouse SOC-1 and SOCS3 and used to probe commercially available mouse thymus and spleen cDNA libraries. Sequencing was performed using an ABI automated sequencer according to the manufacturer's instructions.

**EXAMPLE 7****SOUTHERN AND NORTHERN BLOT ANALYSES AND RT-PCR**

<sup>32</sup>P-labelled probes were generated using a random decanucleotide labelling kit (Bresatec, Adelaide, South Australia) from a 600 bp Pst I fragment encoding neomycin phosphotransferase from the plasmid pPGKneo, 1070 bp fragment of the SOCS1 gene obtained by digestion of the 1.4 kbp PCR product with Xho I, SOCS2, SOCS3, CIS and a 1.2 kbp fragment of the chicken glyceraldehyde 3-phosphate dehydrogenase gene [Dugaiczyk, 1983].

Genomic DNA was isolated from cells using a proteinase K-sodium dodecyl sulfate procedure essentially as described. Fifteen micrograms of DNA was digested with either BamHI or SacI, fractionated on a 0.8%(w/v) agarose gel, transferred to GeneScreenPlus membrane (Du Pont NEN, Boston MA), prehybridised, hybridised with random-primed <sup>32</sup>P-labelled DNA fragments and washed essentially as described [Sambrook, 1989].

Total RNA was isolated from cells and tissues using Trizol Reagent, as recommended by the manufacturer (GibcoBRL, Grand Island, NY). When required polyA+ mRNA was purified essentially as described [Alexander, 1995]. Northern blots were prehybridised, hybridized with random-primed <sup>32</sup>P-labelled DNA fragments and washed as described [Alexander, 1995].

To assess the induction of SOCS genes by IL-6, mice (C57BL6) were injected intravenously

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with 5  $\mu$ g IL-6 followed by harvest of the liver at the indicated timepoints after injection. M1 cells were cultured in the presence of 20 ng/ml IL-6 and harvested at the indicated times. For RT-PCR analysis, bone marrow cells were harvested as described (Metcalf *et al.*, 1995) and stimulated for 1 hr at 37°C with 100 ng/ml of a range of cytokines. RT-PCR was performed  
5 on total RNA as described (Metcalf *et al.*, 1995). PCR products were resolved on an agarose gel and Southern blots were hybridised with probes specific for each SOCS family member. Expression of  $\beta$ -actin was assessed to ensure uniformity of amplification.

### EXAMPLE 8

#### 10 DNA CONSTRUCTS AND TRANSFECTION

A cDNA encoding epitope-tagged SOCS1 was generated by subcloning the entire SOCS1 coding region into the pEF-BOS expression vector [Mizushima, 1990], engineered to encode an inframe FLAG epitope downstream of an initiation methionine (pF-SOCS1). Using electroporation as described previously [Hilton, 1994], M1 cells expressing the thrombopoietin  
15 receptor (M1.mpl) were transfected with the 20  $\mu$ g of Aat II-digested pF-SOCS1 expression plasmid and 2  $\mu$ g of a Sca I-digested plasmid in which transcription of a cDNA encoding puromycin N-acetyl transferase was driven from the mouse phosphoglycerokinase promoter (pPGKpuropA). After 48 hours in culture, transfected cells were selected with 20  $\mu$ g/ml puromycin (Sigma Chemical Company, St Louis MO), and screened for expression of SOCS1  
20 by Western blotting, using the M2 anti-FLAG monoclonal antibody according to the manufacturer's instructions (Eastman Kodak, Rochester NY). In other experiments M1 cells were transfected with only the pF-SOCS1 plasmid or a control and selected by their ability to grow in agar in the presence of 100 ng/ml of IL-6.

25

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## EXAMPLE 9

## IMMUNOPRECIPITATION AND WESTERN BLOTTING

Prior to either immunoprecipitation or Western blotting,  $10^7$  M1 cells or their derivatives were washed twice, resuspended in 1ml of DME, and incubated at 37°C for 30 min. The cells were then stimulated for 4 min at 37°C with either saline or 100 ng/ml IL-6, after which sodium vanadate (Sigma Chemical Co., St Louis, MI) was added to a concentration of 1 mM. Cells were placed on ice, washed once with saline containing 1 mM sodium vanadate, and then solubilised for 5 min on ice with 300 µl 1% (v/v) Triton X-100, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl pH 7.4, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany) and 1 mM sodium vanadate. Lysates were cleared by centrifugation and quantitated using a Coomassie Protein Assay Reagent (Pierce, Rockford IL).

For immunoprecipitations, equal concentrations of protein extracts (1-2 mg) were incubated for 1 hr or overnight at 4°C with either 4 µg of anti-gp130 antibody (M20; Santa Cruz Biotechnology Inc., Santa Cruz, CA) or 4 µg of anti-phosphotyrosine antibody (4G10; Upstate Biotechnology Inc., Lake Placid NY), and 15 µl packed volume of Protein G Sepharose (Pharmacia, Uppsala, Sweden) [Hilton *et al*, 1996]. Immunoprecipitates were washed twice in 1% (v/v) NP40, 150 mM NaCl, 50 mM Tris-HCl pH 8.0, containing Complete protease inhibitors (Boehringer Mannheim, Mannheim, Germany and 1 mM sodium vanadate. The samples were heated for 5 min at 95°C in SDS sample buffer (625 mM Tris-HCl pH 6.8, 0.05% (w/v) SDS, 0.1% (v/v) glycerol, bromophenol blue, 0.125% (v/v) 2-mercaptoethanol), fractionated by SDS-PAGE and immunoblotted as described above.

For Western blotting, 10 µg of protein from a cellular extract or material from an immunoprecipitation reaction was loaded onto 4-15% Ready gels (Bio-Rad Laboratories, Hercules CA), and resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to PVDF membrane (Micron Separations Inc., Westborough MA) for 1 hr at 100 V. The membranes were probed with the following primary antibodies; anti-tyrosine phosphorylated STAT3 (1:1000 dilution; New England Biolabs, Beverly, MA); anti-STAT3 (C-20; 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-gp130 (M20, 1:100 dilution; Santa Cruz Biotechnology Inc., Santa Cruz CA); anti-

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phosphotyrosine (horseradish peroxidase-conjugated RC20, 1:5000 dilution; Transduction Laboratories, Lexington KY); anti-tyrosine phosphorylated MAP kinase and anti-MAP kinase antibodies (1:1000 dilution; New England Biolabs, Beverly, MA). Blots were visualised using peroxidase-conjugated secondary antibodies and Enhanced Chemiluminescence (ECL) reagents  
5 according to the manufacturer's instructions (Pierce, Rockford IL).

### EXAMPLE 10

#### ELECTROPHORETIC MOBILITY SHIFT ASSAYS

Assays were performed as described [Novak, 1995], using the high affinity SIF (c-sis- inducible  
10 factor) binding site m67 [Wakao, 1994]. Protein extracts were prepared from M1 cells incubated for 4-10 min at 37°C in 10 ml serum-free DME containing either saline, 100 ng/ml IL-6 or 100 ng/ml IFN-γ. The binding reactions contained 4-6 µg protein (constant within a given experiment), 5 ng <sup>32</sup>P-labelled m67 oligonucleotide, and 800 ng sonicated salmon sperm DNA. For certain experiments, protein samples were preincubated with an excess of unlabelled  
15 m67 oligonucleotide, or antibodies specific for either STAT1 (Transduction Laboratories, Lexington, KY) or STAT3 (Santa Cruz Biotechnology Inc., Santa Cruz CA), as described [Novak, 1995].

Western blots were performed using anti-tyrosine phosphorylated STAT3 or anti-STAT3 (New  
20 England Biolabs, Beverly, MA) or anti-gp130 (Santa Cruz Biotechnology Inc.) as described (Nicola *et al*, 1996). EMSA were performed using the m67 oligonucleotide probe, as described (Novak *et al*, 1995).

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### EXAMPLE 11

#### EXPRESSION CLONING OF A NOVEL SUPPRESSOR OF CYTOKINE SIGNAL TRANSDUCTION

In order to identify cDNAs capable of suppressing cytokine signal transduction, an expression  
5 cloning approach was adopted. This strategy centred on M1 cells, a monocytic leukaemia cell  
line that differentiates into mature macrophages and ceases proliferation in response to the  
cytokines IL-6, LIF, OSM and IFN- $\gamma$ , and the steroid dexamethasone. Parental M1 cells were  
infected with the RUFneo retrovirus, into which cDNAs from the factor-dependent  
haemopoietic cell line FDC-P1 had been cloned. In this retrovirus, transcription of both the  
10 neomycin resistance gene and the cloned cDNA was driven off the powerful constitutive  
promoter present in the retroviral LTR (Figure 1). When cultured in semi-solid agar, parental  
M1 cells form large tightly packed colonies. Upon stimulation with IL-6, M1 cells undergo  
rapid differentiation, resulting in the formation in agar of only single macrophages or small  
dispersed clusters of cells. Retrovirally-infected M1 cells that were unresponsive to IL-6 were  
15 selected in semi-solid agar culture by their ability to form large, tightly packed colonies in the  
presence of IL-6 and geneticin. A single stable IL-6-unresponsive clone, 4A2, was obtained  
after examining  $10^4$  infected cells.

A fragment of the neomycin phosphotransferase (neo) gene was used to probe a Southern blot  
20 of genomic DNA from clone 4A2 and this revealed that the cell line was infected with a single  
retrovirus containing a cDNA approximately 1.4 kbp in length (Figure 2). PCR amplification  
using primers from the retroviral vector which flanked the cDNA cloning site enabled recovery  
of a 1.4 kbp cDNA insert, which we have named suppressor of cytokine signalling-1, or  
SOCS1. This PCR product was used to probe a similar Southern blot of 4A2 genomic DNA  
25 and hybridised to two fragments, one which corresponded to the endogenous SOCS1 gene and  
the other, which matched the size of the band seen using the neo probe, corresponded to the  
SOCS1 cDNA cloned into the integrated retrovirus (Figure 2). The latter was not observed in  
an M1 cell clone infected with a retrovirus containing an irrelevant cDNA. Similarly, Northern  
blot analysis revealed that SOCS1 mRNA was abundant in the cell line 4A2, but not in the  
30 control infected M1 cell clone (Figure 2).

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**EXAMPLE 12****SOCS1, SOCS2, SOCS3 AND CIS DEFINE A NEW FAMILY  
OF SH2-CONTAINING PROTEINS**

5 The SOCS1 PCR product was used as a probe to isolate homologous cDNAs from a mouse thymus cDNA library. The sequence of the cDNAs proved to be identical to the PCR product, suggesting that constitutive or over expression, rather than mutation, of the SOCS1 protein was sufficient for generating an IL-6-unresponsive phenotype. Comparison of the sequence of SOCS1 cDNA with nucleotide sequence databases revealed that it was present on mouse and  
10 rat genomic DNA clones containing the protamine gene cluster found on mouse chromosome 16. Closer inspection revealed that the 1.4 kb SOCS1 sequence was not homologous to any of the protamine genes, but rather represented a previously unidentified open reading frame located at the extreme 3' end of these clones (Figure 3). There were no regions of discontinuity between the sequences of the SOCS1 cDNA and genomic locus, suggesting that SOCS1 is  
15 encoded by a single exon. In addition to the genomic clone containing the protamine genes, a series of murine and human expressed sequenced tags (ESTs) also revealed large blocks of nucleotide sequence identity to mouse SOCS1. The sequence information provided by the human ESTs allowed the rapid cloning of cDNAs encoding human SOCS1.

20 The mouse and rat SOCS1 gene encodes a 212 amino acid protein whereas the human SOCS1 gene encodes a 211 amino acid protein. Mouse, rat and human SOCS1 proteins share 95-99% amino acid identity (Figure 9). A search of translated nucleic acid databases with the predicted amino acid sequence of SOCS1 showed that it was most related to a recently cloned cytokine-inducible immediate early gene product, CIS, and two classes of ESTs. Full length cDNAs  
25 from the two classes of ESTs were isolated and found to encode proteins of similar length and overall structure to SOCS1 and CIS. These clones were given the names SOCS2 and SOCS3. Each of the four proteins contains a central SH2 domain and a C-terminal region termed the SOCS motif. The SOCS1 proteins exhibit an extremely high level of amino acid sequence similarity (95-99% identity) amongst different species. However, the forms of the SOCS1,  
30 SOCS2, SOCS3 and CIS from the same animal, while clearly defining a new family of SH2-containing proteins, exhibited a lower amino acid identity. SOCS2 and CIS exhibit



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approximately 38% amino acid identity, while the remaining members of the family share approximately 25% amino acid identity (Figure 9). The coding region of the genes for SOCS1 and SOCS3 appear to contain no introns while the coding region of the genes for SOCS2 and CIS contain one and two introns, respectively.

5

The Genbank Accession Numbers for the sequences referred to herein are mouse SOCS1 cDNA (U88325), human SOCS1 cDNA (U88326), mouse SOCS2 cDNA (U88327), mouse SOCS3 cDNA (U88328).

10

### EXAMPLE 13

#### CONSTITUTIVE EXPRESSION OF SOCS1 SUPPRESSES THE ACTION OF A RANGE OF CYTOKINES

To formally establish that the phenotype of the 4A2 cell line was directly related to expression of SOCS1, and not to unrelated genetic changes which may have occurred independently in  
15 these cells, a cDNA encoding an epitope-tagged version of SOCS1 under the control of the EF1 $\alpha$  promoter was transfected into parental M1 cells, and M1 cells expressing the receptor for thrombopoietin, c-mpl (M1.mpl). Transfection of the SOCS1 expression vector into both cell lines resulted in an increase in the frequency of IL-6 unresponsive M1 cells.

20 Multiple independent clones of M1 cells expression SOCS1, as detected by Western blot, displayed a cytokine-unresponsive phenotype that was indistinguishable from 4A2. Further, if transfectants were not maintained in puromycin, expression of SOCS1 was lost over time and cells regained their cytokine responsiveness. In the absence of cytokine, colonies derived from 4A2 and other SOCS1 expressing clones characteristically grew to a smaller size than colonies  
25 formed by control M1 cells (Figure 10).

The effect of constitutive SOCS1 expression on the response of M1 cells to a range of cytokines was investigated using the 4A2 cell line and a clone of M1.mpl cells expressing SOCS1 (M1.mpl.SOCS1). Unlike parental M1 cells and M1.mpl cells, the two cell lines  
30 expressing SOCS1 continued to proliferate and failed to form differentiated colonies in response to either IL-6, LIF, OSM, IFN- $\gamma$  or, in the case of the M1.mpl.SOCS1 cell line, thrombopoietin

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(Figure 4). For both cell lines, however, a normal response to dexamethasone was observed, suggesting that SOCS1 specifically affected cytokine signal transduction rather than differentiation *per se*. Consistent with these data, while parental M1 cells and M1.mpl cells became large and vacuolated in response to IL-6, 4A2 and M1.mpl.SOCS1 cells showed no  
5 evidence of morphological differentiation in response to IL-6 or other cytokines (Figure 5).

#### EXAMPLE 14

##### SOCS1 INHIBITS A RANGE OF IL-6 SIGNAL TRANSDUCTION PROCESSES, INCLUDING STAT3 PHOSPHORYLATION

##### 10 AND ACTIVATION

Phosphorylation of the cell surface receptor component gp130, the cytoplasmic tyrosine kinase JAK1 and the transcription factor STAT3 is thought to play a central role in IL-6 signal transduction. These events were compared in the parental M1 and M1.mpl cell lines and their SOCS1-expressing counterparts. As expected, gp130 was phosphorylated rapidly in response  
15 to IL-6 in both parental lines, however, this was reduced five- to ten-fold in the cell lines expressing SOCS1 (Figure 6). Likewise, STAT3 phosphorylation was also reduced by approximately ten-fold in response to IL-6 in those cell lines expressing SOCS1 (Figure 6). Consistent with a reduction in STAT3 phosphorylation, activation of specific STAT DNA binding complexes, as determined by electrophoretic mobility shift assay, was also reduced.  
20 Notably, there was a reduction in the formation of SIF-A (containing STAT3), SIF-B (STAT1/STAT3 heterodimer) and SIF-C (containing STAT1), the three STAT complexes induced in M1 cells stimulated with IL-6 (Figure 7). Similarly, constitutive expression of SOCS1 also inhibited IFN- $\gamma$ -stimulated formation of p91 homodimers (Figure 7). STAT phosphorylation and activation were not the only cytoplasmic processes to be effected by  
25 SOCS1 expression, as the phosphorylation of other proteins, including shc and MAP kinase, was reduced to a similar extent (Figure 7).

#### EXAMPLE 15

##### TRANSCRIPTION OF THE SOCS1 GENE IS STIMULATED BY IL-6

##### 30 *IN VITRO AND IN VIVO*

Although SOCS1 can inhibit cytokine signal transduction when constitutively expressed in M1

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cells, this does not necessarily indicate that SOCS1 normally functions to negatively regulate an IL-6 response. In order to investigate this possibility the inventors determined whether transcription of the SOCS1 gene is regulated in the response of M1 cells to IL-6 and, because of the critical role IL-6 plays in regulating the acute phase response to injury and infection, the response of the liver to intravenous injection of 5 mg IL-6. In the absence of IL-6, SOCS1 mRNA was undetectable in either M1 cells or in the liver. However, for both cell types, a 1.4 kb SOCS1 transcript was induced within 20 to 40 minutes by IL-6 (Figure 8). For M1 cells, where the IL-6 was present throughout the experiment, the level of SOCS1 mRNA remained elevated (Figure 8). In contrast, IL-6 was administered in vivo by a single intravenous injection and was rapidly cleared from the circulation, resulting in a pulse of IL-6 stimulation to the liver. Consistent with this, transient expression of SOCS1 mRNA was detectable in the liver, peaking approximately 40 minutes after injection and declining to basal levels within 4 hours (Figure 8).

#### EXAMPLE 16

#### REGULATION OF SOCS GENES

Since CIS was cloned as a cytokine-inducible immediate early gene the inventors examined whether SOCS1, SOCS2 and SOCS3 were similarly regulated. The basal pattern of expression of the four SOCS genes was examined by Northern blot analysis of mRNA from a variety of tissues from male and female C57B1/6 mice (Figure 11A). Constitutive expression of SOCS1 was observed in the thymus and to a lesser extent in the spleen and the lung. SOCS2 expression was restricted primarily to the testis and in some animals the liver and lung; for SOCS3 a low level of expression was observed in the lung, spleen and thymus, while CIS expression was more widespread, including the testis, heart, lung, kidney and, in some animals, the liver.

The inventors sought to determine whether expression of the four SOCS genes was regulated by IL-6. Northern blots of mRNA prepared from the livers of untreated and IL-6-injected mice, or from unstimulated and IL-6-stimulated M1 cells, were hybridised with labelled fragments of SOCS1, SOCS2, SOCS3 and CIS cDNAs (Figure 11B). Expression of all four SOCS genes was increased in the liver following IL-6 injection, however the kinetics of

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induction appeared to differ. Expression of SOCS1 and SOCS3 was transient in the liver, with mRNA detectable after 20 minutes of IL-6 injection and declining to basal levels within 4 hours for SOCS and 8 hours for SOCS3. Induction of SOCS2 and CIS mRNA in the liver followed similar initial kinetics to that of SOCS1, but was maintained at an elevated level for at least 24 hours. A similar induction of SOCS gene mRNA was observed in other organs, notably the lung and the spleen. In contrast, in M1 cells, while SOCS1 and CIS mRNA were induced by IL-6, no induction of either SOCS2 or SOCS3 expression was detected. This result highlights cell type-specific differences in the expression of the genes of SOCS family members in response to the same cytokine.

10

In order to examine the spectrum of cytokines that was capable of inducing transcription of the various members of the SOCS gene family, bone marrow cells were stimulated for an hour with a range of cytokines, after which mRNA was extracted and cDNA was synthesised. PCR was then used to assess the expression of SOCS1, SOCS2, SOCS3 and CIS (Figure 11C). In the absence of stimulation, little or no expression of any of the SOCS genes was detectable in bone marrow by PCR. Stimulation of bone marrow cells with a broad array of cytokines appeared capable of up regulating mRNA for one or more members of the SOCS family. IFN $\gamma$ , for example, induced expression of all four SOCS genes, while erythropoietin, granulocyte colony-stimulating factor, granulocyte-macrophage colony stimulating factor and interleukin-3 induced expression of SOCS2, SOCS3 and CIS. Interestingly, tumor necrosis factor alpha, macrophage colony-stimulating factor and interleukin-1, which act through receptors that do not fall into the type I cytokine receptor class also appeared capable of inducing expression of SOCS3 and CIS, suggesting that SOCS proteins may play a broader role in regulating signal transduction.

25 As constitutive expression of SOCS1 inhibited the response of M1 cells to a range of cytokines, the inventors examined whether phosphorylation of the cell surface receptor component gp130 and the transcription factor STAT3, which are thought to play a central role in IL-6 signal transduction, were affected. These events were compared in the parental M1 and M1.mpl cell lines and their SOCS1-expressing counterparts. As expected, gp130 was phosphorylated rapidly in response to IL-6 in both parental lines, however, this was reduced in the cell lines expressing SOCS1 (Figure 12A). Likewise, STAT3 phosphorylation was also reduced in

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response to IL-6 in those cell lines expressing SOCS1 (Figure 12A). Consistent with a reduction in STAT3 phosphorylation, activation of specific STAT/DNA binding complexes, as determined by electrophoretic mobility shift assay, was also reduced. Notably, there was a failure to form SIF-A (containing STAT3) and SIF-B(STAT1/STAT3 heterodimer), the major  
5 STAT complexes induced in M1 cells stimulated with IL-6 (Figure 12B). Similarly, constitutive expression of SOCS1 also inhibited IFN $\gamma$ -stimulating formation of SIF-C (STAT1 homodimer; Figure 12B). These experiments are consistent with the proposal that SOCS1 inhibits signal transduction upstream of receptor and STAT phosphorylation, potentially at the level of the JAK kinases.

10

The ability of SOCS1 to inhibit signal transduction and ultimately the biological response to cytokines suggest that, like the SH2-containing phosphatase SHP-1 [Ihle *et al.*, 1994; Yi *et al.*, 1993], the SOCS proteins may play a central role in controlling the intensity and/or duration of a cell's response to a diverse range of extracellular stimuli by suppressing the signal  
15 transduction process. The evidence provided here indicates that the SOCS family acts in a classical negative feedback loop for cytokine signal transduction. Like other genes such as OSM, expression of genes encoding the SOCS proteins is induced by cytokines through the activation of STATs. Once expressed, it is proposed that the SOCS proteins inhibit the activity of JAKs and so reduce the phosphorylation of receptors and STATs, thereby suppressing signal  
20 transduction and any ensuing biological response. Importantly, inhibition of STAT activation will, over time, lead to a reduction in SOCS gene expression, allowing cells to regain responsiveness to cytokines.

#### EXAMPLE 17

25

#### DATABASE SEARCHES

The NCBI genetic sequence database (Genbank), which encompasses the major database of expressed sequence tags (ESTs) and TIGR database of human expressed sequence tags, were searched for sequences with similarity to a consensus SOCS box sequence using the TFASTA  
30 and MOTIF/PATTERN algorithms [Pearson, 1990; Cockwell and Giles, 1989]. Using the software package SRS [Etzold *et al.*, 1996], ESTs that exhibited similarity to the SOCS box

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(and their partners derived from sequencing the other end of cDNAs) were retrieved and assembled into contigs using Autoassembler (Applied Biosystems, Foster City, CA). Consensus nucleotide sequences derived from overlapping ESTs were then used to search the various databases using BLASTN [Altschul *et al*, 1990]. Again, positive ESTs were retrieved and  
 5 added to the contig. This process was repeated until no additional ESTs could be recovered. Final consensus nucleotide sequences were then translated using Sequence Navigator (Applied Biosystems, Foster City, CA).

The ESTs encoding the new SOCS proteins are as follows: **human SOCS4** (EST81149,  
 10 EST180909, EST182619, ya99H09, yc70co4, yh53c09, yh77g11, yh87h05, yi45h07, yj04e06, yq12h06, yq56a06, yq60e02, yq92g03, yq97h06, yr90f01, yt69c03, yv30a08, yv55f07, yv57h09, yv87h02, yv98e11, yw68d10, yw82a03, yx08a07, yx72h06, yx76b09, yy37h08, yy66b02, za81f08, zb18f07, zc06e08, zd14g06, zd51h12, zd52b09, ze25g11, ze69f02, zf54f03, zh96e07, zv66h12, zs83a08 and zs83g08). **mouse SOCS-4** (mc65f04, mf42e06, mp10c10,  
 15 mr81g09, and mt19h12). **human SOCS-5** (EST15B103, EST15B105, EST27530 and zf50f01). **mouse SOCS-5** (mc55a01, mh98f09, my26h12 and ve24e06). **human SOCS-6** (yf61e08, yf93a09, yg05f12, yg41f04, yg45c02, yh11f10, yh13b05, zc35a12, ze02h08, zl09a03, zl69e10, zn39d08 and zo39e06). **mouse SOCS-6** (mc04c05, md48a03, mf31d03, mh26b07, mh78e11, mh88h09, mh94h07, mi27h04 and mj29c05, mp66g04, mw75g03, va53b05,  
 20 vb34h02, vc55d07, vc59e05, vc67d03, vc68d10, vc97h01, vc99c08, vd07h03, vd08c01, vd09b12, vd19b02, vd29a04 and vd46d06). **human SOCS-7** (STS WI30171, EST00939, EST12913, yc29b05, yp49f10, zt10f03 and zx73g04). **mouse SOCS-7** (mj39a01 and vi52h07). **mouse SOCS-8** (mj6e09 and vj27a029). **human SOCS-9** (CSRL-82f2-u, EST114054, yy06b07, yy06g06, zr40c09, zr72h01, yx92c08, yx93b08 and hfe0662). **mouse**  
 25 **SOCS-9** (me65d05). **human SOCS-10** (aa48h10, zp35h01, zp97h12, zq08b01, zr34g05, EST73000 and HSDHEI005). **mouse SOCS-10** (mb14d12, mb40f06, mg89b11, mq89e12, mp03g12 and vh53c11). **human SOCS-11** (zt24h06 and zr43b02). **human SOCS-13** (EST59161). **mouse SOCS-13** (ma39a09, me60c05, mi78g05, mk10c11, mo48g12, mp94a01, vb57c07 and vh07c11). **human SOCS-14** (mi75e03, vd29h11 and vd53g07).

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### EXAMPLE 18

#### cDNA CLONING

Based on the concensus sequences derived from overlapping ESTs, oligonucleotides were  
5 designed that were specific to various members of the SOCS family. As described above,  
oligonucleotides were labelled and used to screen commercially available genomic and cDNA  
libraries cloned with  $\lambda$  bacteriophage. Genomic and/or cDNA clones covering the entire coding  
region of mouse SOCS4, mouse SOCS5 and mouse SOCS6 were isolated. The entire gene for  
SOCS15 is on the human 12p13 BAC (Genbank Accession Number HSU47924) and the mouse  
10 chromosome 6 BAC (Genbank Accession Number AC002393). Partial cDNAs for mouse  
SOCS7, SOCS9, SOCS10, SOCS11, SOCS12, SOCS13 and SOCS14 were also isolated.

### EXAMPLE 19

#### NORTHERN BLOTS AND rtPCR

15 Northern blots were performed as described above. The sources of hybridisation probes were  
as follows; (i) the entire coding region of the mouse SOCS1 cDNA, (ii) a 1059 bp PCR product  
derived from coding region of SOCS5 upstream of the SH2 domain, (iii) the entire coding  
region of the mouse SOCS6 cDNA, (iv) a 790 bp PCR product derived from the coding region  
20 of a partial SOCS7 cDNA and (v) a 1200 bp Pst I fragment of the chicken glyceraldehyde 3-  
phosphate dehydrogenase (GAPDH) cDNA.

### EXAMPLE 20

#### ADDITIONAL MEMBERS OF SOCS FAMILY

25 SOCS1, SOCS2 and SOCS3 are members of the SOCS protein family identified in Examples  
1-16. Each contains a central SH2 domain and a conserved motif at the C-terminus, named the  
SOCS box. In order to isolate further members of this protein family, various DNA databases  
were searched with the amino acid sequence corresponding to conserved residues of the SOCS  
30 box. This search revealed the presence of human and mouse ESTs encoding twelve further  
members of the SOCS protein family (Figure 13). Using this sequence information cDNAs

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encoding SOCS4, SOCS5, SOCS6, SOCS7, SOCS9, SOCS10, SOCS11, SOCS12, SOCS13, SOCS14 and SOCS15 have been isolated. Further analysis of contigs derived from ESTs and cDNAs revealed that the SOCS proteins could be placed into three groups according to their predicted structure N-terminal of the SOCS box. The three groups are those with (i) SH2 domains, (ii) WD-40 repeats and (iii) ankyrin repeats.

10

#### EXAMPLE 21

##### SOCS PROTEIN WITH SH2 DOMAINS

Eight SOCS proteins with SH2 domains have been identified. These include SOCS1, SOCS2 and SOCS3, SOCS5, SOCS9, SOCS11 and SOCS14 (Figure 13). Full length cDNAs were isolated for mouse SOCS5 and SOCS14 and partial clones encoding mouse SOCS9 and SOCS14. Analysis of primary amino acid sequence and genomic structure suggest that pairs of these proteins (SOCS1 and SOCS3, SOCS2 and CIS, SOCS5 and SOCS14 and SOCS9 and SOCS11) are most closely related (Figure 13). Indeed, the SH2 domains of SOCS5 and SOCS14 are almost identical (Figure 13B), and unlike CIS, SOCS1, SOCS2 and SOCS3, SOCS5 and SOCS14 have an extensive, though less well conserved, N-terminal region preceding their SH2 domains (Figure 13A).

20

#### EXAMPLE 22

##### SOCS PROTEINS WITH WD-40 REPEATS

25

Four SOCS proteins with WD-40 repeats were identified. As with the SOCS proteins with SH2 domains, pairs of these proteins appeared to be closely related. Full length cDNAs of mouse SOCS4 and SOCS6 were isolated and shown to encode proteins containing eight WD-40 repeats N-terminal of the SOCS box (Figure 13) and SOCS4 and SOCS6 share 65% amino acid similarity. SOCS15 was recognised as an open reading frame upon sequencing BACs from

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human chromosome 12p13 and the syntenic region of mouse chromosome 6 [Ansari-Lari *et al*, 1997]. In the human, chimp and mouse, SOCS15 is encoded by a gene with two coding exons that lies within a few hundred base pairs of the 3' end of the triose phosphate isomerase (TPI) gene, but which is encoded on the opposite strand to TPI (9). In addition to a C-terminal  
5 SOCS box, the SOCS15 protein contains four WD-40 repeats. Interestingly, within the EST databases, there is a sequence of a nematode, an insect and a fish relative of SOCS15. SOCS15 appears most closely related to SOCS13.

### EXAMPLE 23

10

#### SOCS PROTEINS WITH ANKYRIN REPEATS

Three SOCS proteins with ankyrin repeats were identified. Analysis of partial cDNAs of mouse SOCS7, SOCS10 and SOCS12 demonstrated the presence of multiple ankyrin repeats.

15

### EXAMPLE 24

#### EXPRESSION PATTERN OF SOCS PROTEINS

The expression of mRNA from representative members of each class of SOCS proteins - SOCS1 and SOCS5 from the SH2 domain group, SOCS6 from the WD-40 repeat group and  
20 SOCS7 from the ankyrin repeat group was examined. As shown above, SOCS1 mRNA is found in abundance in the thymus and at lower levels in other adult tissues.

Since transcription of the SOCS1 gene is induced by cytokines, the inventors sought to determine whether levels of SOCS5, SOCS6 and SOCS7 mRNA increased upon cytokine  
25 stimulation. In the livers of mice injected with IL-6, SOCS1 mRNA is detectable after 20 min and decreases to background levels within 2 hours. In contrast, the kinetics of SOCS5 mRNA expression are quite different, being only detectable 12 to 24 hours after IL-6 injection. SOCS6 mRNA appears to be expressed constitutively while SOCS7 mRNA was not detected in the liver either before injection of IL-6 or at any time after injection.

30

Expression of these genes was also examined after cytokine stimulation of the factor-dependent

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cell line FDCP-1 engineered to express bcl-w. Again, while SOCS6 mRNA was expressed constitutively.

#### EXAMPLE 25

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#### SOCS4

Mouse and human SOCS4 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS4 cDNAs are tabulated below (Tables 4.1 and 4.2). Using sequence information derived from mouse ESTs  
10 several oligonucleotides were designed and used to screen, in the conventional manner, a mouse thymus cDNA library cloned into  $\lambda$ -bacteriophage. Two cDNAs encoding mouse SOCS4 were isolated and sequenced in their entirety (Figure 15) and shown to overlap the mouse ESTs identified in the database (Table 4.1 and Figure 17). These cDNAs include a region of 5' untranslated region, the entire mouse SOCS4 coding region and a region of 3' untranslated  
15 region (Figure 17). Analysis of the sequence confirms that the SOCS4 cDNA encodes a SOCS Box at its C-terminus and a series of 8 WD-40 repeats before the SOCS Box (Figures 17 and 16). The relationship of the two sequence contigs of human SOCS4 (h4.1 and h4.2) to the experimentally determined mouse SOCS4 cDNA sequence is shown in Figure 17. The nucleotide sequence of the two human contigs is listed in Figure 18.

20

SEQ ID NO:13 and 14 represent the nucleotide sequence of murine SOCS4 and the corresponding amino acid sequence. SEQ ID NOs: 15 and 16 are SOCS4 cDNA human contigs h4.1 and h4.2, respectively.

25

#### EXAMPLE 26

#### SOCS5

Mouse and human SOCS5 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS5 cDNAs are  
30 tabulated below (Tables 5.1 and 5.2). Using sequence information derived from mouse and human ESTs, several oligonucleotides were designed and used to screen, in the conventional

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manner, a mouse thymus cDNA library, a mouse genomic DNA library and a human thymus cDNA library cloned into  $\lambda$ -bacteriophage. A single genomic DNA clone (57-2) and (5-3-2) cDNA clone encoding mouse SOCS5 were isolated and sequenced in their entirety and shown to overlap with the mouse ESTs identified in the database (Figures 19 and 20A). The entire coding region, in addition to a region of 5' and 3' untranslated regions of mouse SOCS5 appears to be encoded on a single exon (Figure 19). Analysis of the sequence (Figure 20) confirms that SOCS5 genomic and cDNA clones encode a protein with a SOCS box at its C-terminus in addition to an SH2 domain (Figure 19 and 20B). The relationship of the human SOCS5 contig (h5.1; Figure 21) derived from analysis of cDNA clone 5-94-2 and the human SOCS5 ESTs (Table 5.2) to the mouse SOCS5 DNA sequence is shown in Figure 19. The nucleotide sequence and corresponding amino acid sequence of murine SOCS5 are shown in SEQ ID NOs: 17 and 18, respectively. The human SOCS5 nucleotide sequence is shown in SEQ ID NO:19.

15

**EXAMPLE 27****SOCS6**

Mouse and human SOCS6 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS6 cDNAs are tabulated below (Tables 6.1 and 6.2). Using sequence information derived from mouse ESTs, several oligonucleotides were designed and used to screen, in the conventional manner, a mouse thymus cDNA library. Eight cDNA clones (6-1A, 6-2A, 6-5B, 6-4N, 6-18, 6-29, 6-3N, 6-5N) cDNA clone encoding mouse SOCS6 were isolated and sequenced in their entirety and shown to overlap with the mouse ESTs identified in the database (Figures 22 and 23A). Analysis of the sequence (Figure 23) confirms that the mouse SOCS6 cDNA clones encode a protein with a SOCS box at its C-terminus in addition to a eight WD-40 repeats (Figures 22 and 23B). The relationship of the human SOCS-6 contigs (h6.1 and h6.2 ; Figure 24) derived from analysis of human SOCS6 ESTs (Table 6.2) to the mouse SOCS6 DNA sequence is shown in Figure 22. The nucleotide and corresponding amino acid sequences of murine SOCS6 are shown in SEQ ID NOs: 20 and 21, respectively. SOCS6 human contigs h6.1 and h6.2 are shown in SEQ ID NOs: 22 and 23, respectively.

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**EXAMPLE 28****SOCS7**

Mouse and human SOCS7 were recognized through searching EST databases using the SOCS  
5 box consensus (Figure 13). Those ESTs derived from mouse and human SOCS-7 cDNAs are  
tabulated below (Tables 7.1 and 7.2). Using sequence information derived from mouse ESTs,  
several oligonucleotides were designed and use to screen, in the conventional manner, a mouse  
thymus cDNA library. One cDNA clone (74-10A-11) cDNA clone encoding mouse SOCS7  
was isolated and sequenced in its entirety and shown to overlap with the mouse ESTs identified  
10 in the database (Figures 25 and 26A). Analysis of the sequence (Figure 26) suggests that  
mouse SOCS7 encodes a protein with a SOCS box at its C-terminus, in addition to several  
ankyrin repeats (Figure 25 and 26B). The relationship of the human SOCS7 contigs (h7.1 and  
h7.2 ; Figure 27) derived from analysis of human SOCS7 ESTs (Table 7.2) to the mouse  
SOCS7 DNA sequence is shown in Figure 25. The nucleotide and corresponding amino acid  
15 sequences of murine SOCS7 are shown in SEQ ID NOs: 24 and 25, respectively. The  
nucleotide sequence of SOCS7 human contigs h7.1 and h7.2 are shown in SEQ ID NOs: 26 and  
27, respectively.

**EXAMPLE 29**

20

**SOCS8**

ESTs derived from mouse SOCS8 cDNAs are tabulated below (Table 8.1). As described for  
other members of the SOCS family, it is possible to isolate cDNAs for mouse SOCS8 using  
sequence information derived from mouse ESTs. The relationship of the ESTs to the predicted  
25 coding region of SOCS8 is shown in Figure 28. With the nucleotide sequence obtained from  
the ESTs shown in Figure 29A and the partial amino acid sequence of SOCS8 shown in Figure  
29B. The nucleotide sequence and corresponding amino acid sequences for murine SOCS8 are  
shown in SEQ ID NOs:28 and 29, respectively.

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**EXAMPLE 30****SOCS9**

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Mouse and human SOCS-9 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS9 cDNAs are tabulated below (Tables 9.1 and 9.2). The relationship of the mouse SOCS9 contigs (m9.1; Figure 9.2) derived from analysis of the mouse SOCS9 EST (Table 9.1) to the human SOCS-9 DNA contig (h9.1; Figure 32) derived from analysis of human SOCS9 ESTs (Table 9.2) is shown in Figure 31. Analysis of the sequence (Figure 32) indicates that the human SOCS9 cDNA encodes a protein with a SOCS box at its C-terminus, in addition to an SH2 domain (Figure 30). The nucleotide sequence of murine SOCS9 cDNA is shown in SEQ ID NO:30. The nucleotide sequence of human SOCS9 cDNA is shown in SEQ ID NO:31.

10

### EXAMPLE 31

#### SOCS10

Mouse and human SOCS10 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS10 cDNAs are tabulated below (Table 10.1 and 10.2). Using sequence information derived from mouse ESTs, several oligonucleotides were designed and used to screen, in the conventional manner, a mouse thymus cDNA library. Four cDNA clones (10-9, 10-12, 10-23 and 10-24) encoding mouse SOCS10 were isolated, sequenced in their entirety and shown to overlap with the mouse and human ESTs identified in the database (Figures 33 and 34). Analysis of the sequence (Figure 34) indicates that the mouse SOCS10 cDNA clone is not full length but that it does encode a protein with a SOCS box at its C-terminus, in addition to several ankyrin repeats (Figure 33). The relationship of the human SOCS10 contigs (h10.1 and h10.2 ; Figure 35) derived from analysis of human SOCS10 ESTs (Table 10.2) to the mouse SOCS10 DNA sequence is shown in Figure 33. Comparison of mouse cDNA clones and ESTs with human ESTs suggests that the 3' untranslated regions of mouse and human SOCS10 differ significantly. The nucleotide sequence of murine SOCS10 is shown in SEQ ID NO:32 and the nucleotide sequence of SOCS10 human contigs h10.1 and h10.2 are shown in SEQ ID NOs:33 and 34, respectively.

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### EXAMPLE 32

#### SOCS11

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Human SOCS11 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from human SOCS11 cDNAs are tabulated below (Table 11.1 and 11.2). The relationship of the human SOCS11 contigs (h11.1; Figure 36A, B), derived from analysis ESTs (Table 11.2) to the predicted encoded protein, is shown in Figure 37. Analysis of the sequence indicates that the human SOCS11 cDNA encodes a protein with a SOCS box at its C-terminus, in addition to an SH2 domain (Figure 37 and 36B). The nucleotide sequence and corresponding amino acid sequence of human SOCS11 are represented in SEQ ID NOs:35 and 36, respectively.

### EXAMPLE 33

#### SOCS12

Mouse and human SOCS-12 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS12 cDNAs are tabulated below (Tables 12.1 and 12.2). Using sequence information derived from mouse ESTs, several oligonucleotides were designed and use to screen, in the conventional manner, a mouse thymus cDNA library. Four cDNA clones (10-9, 10-12, 10-23 and 10-24) encoding mouse SOCS12 were isolated, sequenced in their entirety and shown to overlap with the mouse and human ESTs identified in the database (Figures 38 and 39). Analysis of the sequence (Figure 39 and 40) indicates that the SOCS12 cDNA clone encodes a protein with a SOCS box at its C-terminus, in addition to several ankyrin repeats (Figure 38). The relationship of the human SOCS12 contigs (h12.1 and h12.2 ; Figure 40) derived from analysis of human SOCS12 ESTs (Table 12.2) to the mouse SOCS12 DNA sequence is shown in Figure 38. Comparison of mouse cDNA clones and ESTs with human ESTs suggests that the 3' untranslated regions of mouse and human SOCS12 differ significantly. The nucleotide sequence of SOCS12 is shown in SEQ ID NO:37. The nucleotide sequence of human SOCS12 contigs h12.1 and h12.2 are shown in SEQ ID NOs:38 and 39, respectively.

### EXAMPLE 34

#### SOCS13

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Mouse and human SOCS-13 were recognized through searching EST databases using the SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS13 cDNAs are tabulated below (Tables 13.1 and 13.2). Using sequence information derived from mouse ESTs, several oligonucleotides were designed and use to screen, in the conventional  
5 manner, a mouse thymus and a mouse embryo cDNA library. Three cDNA clones (62-1, 62-6-7 and 62-14) encoding mouse SOCS13 were isolated, sequenced in their entirety and shown to overlap with the mouse ESTs identified in the database (Figure 41 and 42A). Analysis of the sequence (Figure 42) indicates that the mouse SOCS13 cDNA encodes a protein with a SOCS box at its C-terminus, in addition to a potential WD-40 repeat (Figure 41 and 42B). The  
10 relationship of the human SOCS13 contigs (h13.1 and h13.2 ; Figure 43) derived from analysis of human SOCS13 ESTs (Table 13.2) to the mouse SOCS13 DNA sequence is shown in Figure 41. The nucleotide sequence and corresponding amino acid sequence of murine SOCS13 and shown in SEQ ID NOs:40 and 41, respectively. The nucleotide sequence of human SOCS13 contig h13.1 is shown in SEQ ID NO:42.

15

### EXAMPLE 35

#### SOCS14

Mouse and human SOCS-14 were recognized through searching EST databases using the  
20 SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS14 cDNAs are tabulated below (Tables 14.1 and 14.2). Using sequence information derived from mouse and human ESTs, several oligonucleotides were designed and use to screen, in the conventional manner, a mouse thymus cDNA library, a mouse genomic DNA library and a human thymus cDNA library cloned into  $\lambda$ -bacteriophage . A single genomic DNA clone (57-  
25 2) and (5-3-2) cDNA clone encoding mouse SOCS14 were isolated and sequenced in their entirety and shown to overlap with the mouse ESTs identified in the database (Figures 44 and 45A). The entire coding region, in addition to a region of 5' and 3' untranslated regions, of mouse SOCS14 appears to be encoded on a single exon (Figure 44). Analysis of the sequence (Figure 45) confirms that SOCS14 genomic and cDNA clones encode a protein with a SOCS  
30 box at its C-terminus in addition to an SH2 domain (Figure 44 and 45B). The relationship of the human SOCS14 contig (h14.1; Figure 14.3) derived from analysis of cDNA clone 5-94-2

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and the human SOCS14 ESTs (Table 14.2) to the mouse SOCS14 DNA sequence is shown in Figure 44.

The nucleotide sequence and corresponding amino acid sequence of murine SOCS14 are  
5 shown in SEQ ID NOs: 43 and 44, respectively.



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**EXAMPLE 36****SOCS15**

Mouse and human SOCS15 were recognized through searching DNA databases using the  
5 SOCS box consensus (Figure 13). Those ESTs derived from mouse and human SOCS15  
cDNAs are tabulated below (Tables 15.1 and 15.2), as are a mouse and human BAC that  
contain the entire mouse and human SOCS-15 genes. Using sequence information derived from  
the ESTs and the BACs it is possible to predict the entire amino acid sequence of SOCS15 and  
as described for the other SOCS genes it is feasible to design specific oligonucleotide probes  
10 to allow cDNAs to be isolated. The relationship of the BACs to the ESTs is shown in Figure  
46 and the nucleotide and predicted amino acid sequence of the SOCS-15, derived from the  
mouse and human BACs is shown in Figures 47 and 48. The nucleotide sequence and  
corresponding amino acid sequence of murine SOCS15 are shown in SEQ ID NOs:46 and 47,  
respectively. The nucleotide and corresponding amino acid sequence of human SOCS15 are  
15 shown in SEQ ID NO:48 and 49, respectively.

**EXAMPLE 37****SOCS INTERACTION WITH JAK2 KINASE**

20 These Examples show interaction between SOCS and JAK2 kinase. Interaction is mediated *via*  
the SH2 domain of SOCS1, 2, 3 and CIS. The interaction resulted in inhibition of JAK2 kinase  
activity by SOCS1 (Figure 49). General interaction between JAK2 and SOCS1, 2, 3, and CIS  
is shown in Figure 50.

25 The following methods are employed:

**Immunoprecipitation:** Cos 6 cells were transiently transfected by electroporation and cultured  
for 48 hours. Cells were then lysed on ice in lysis buffer (50 mM Tris/HCL, pH 7.5, 150 mM  
NaCl, 1% v/v Triton-X-100, 1 mM EDTA, 1 mM Naf, 1 mM Na<sub>3</sub>VO<sub>4</sub>) with the addition of  
30 complete protease inhibitors (Boehringer Mannheim), centrifuged at 4°C (14,000 x g, 10 min)  
and the supernatant retained for immunoprecipitation. JAK2 proteins were immunoprecipitated

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using 5  $\mu$ l anti-JAK2 antibody (UBI). Antigen-antibody complexes were recovered using protein A-Sepharose (30  $\mu$ l of a 50% slurry).

**Western blotting:** Immunoprecipitates were analysed by sodium dodecyl sulphate (SDS) -  
 5 polyacrylamide gel electrophoresis (PAGE) under reducing conditions. Protein was then  
 electrophoretically transferred to nitrocellulose, blocked overnight in 10% w/v skim-milk and  
 washed in PBS/0.1% v/v Tween-20 (Sigma) (wash buffer) prior to incubation with either anti-  
 phosphotyrosine antibody (4G10) (1:5000, UBI), anti-FLAG antibody (1.6  $\mu$ g/ml) or anti-JAK2  
 antibody (1:2000, UBI) diluted in wash buffer/1% w/v BSA for 2 hr. Nitrocellulose blots were  
 10 washed and primary antibody detected with either peroxidase-conjugated sheep anti-rabbit  
 immunoglobulin (1:5000, Silenus) or peroxidase-conjugated sheep anti-mouse immunoglobulin  
 (1:5000, Silenus) diluted in wash buffer/1% w/v BSA. Blots were washed and antibody binding  
 visualised using the enhanced chemiluminescence (ECL) system (Amersham, UK) according  
 to the manufacturers' instructions.

15

***In-vitro* kinase assay:** An *in vitro* kinase assay was performed to assess intrinsic JAK2 kinase  
 catalytic activity. JAK2 protein were immunoprecipitated as described, washed twice in kinase  
 assay buffer (50 mM NaCl, 5 mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 1 mM NaF, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 10 mM  
 HEPES, pH 7.4) and suspended in an equal volume of kinase buffer containing 0.25  $\mu$ Ci/ml ( $\gamma$ -  
 20 <sup>32</sup>P)-ATP (30 min, room temperature). Excess ( $\gamma$ - P)-ATP was removed and the  
 immunoprecipitates analysed by SDS/PAGE under reducing conditions. Gels were subjected  
 to a mild alkaline hydrolysis by treatment with 1 M KOH (55°C, 2 hours) to remove  
 phosphoserine and phosphothreonine. Radioactive bands were visualised with IMAGEQUANT  
 software on a PhosphorImage system (Molecular Dynamics, Sunnyvale, CA, USA).

25

### EXAMPLE 38

#### MAKING SOCS-1 KNOCKOUT CONSTRUCTS

Diagrams of plasmid constructs and knockout constructs are shown in Figures 51-53. The  
 30 genomic SOCS-1 clone 95-11-10 was digested with the restriction enzymes BamHI and EcoRI  
 to obtain a 3.6Kb DNA fragment 3' of the coding region (SOCS-1 exon), which was used as

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the 3' arm in the SOCS-1 knockout vectors. The ends of this fragment were then blunted. This fragment was then ligated into the following vectors:

pBgalpAloxNeo

and pBgalpAloxNeoTK

- 5 which had been linearized at the unique XhoI site and then blunted. This ligation resulted in the formation of the following vectors:

3'SOCS-1 arm in pBgalpAloxNeo

and 3'SOCS-1 arm in pBgalpAloxNeoTK

- 10 The 5' arm of the SOCS-1 knockout vectors was constructed by using PCR to generate a 2.5Kb PCR product from the genomic SOCS-1 clone 95-11-10 just 5' of the SOCS-1 coding region (SOCS-1 exon). The oligo's used to generate this product were:

5' oligo (sense) (2465)

AGCT AGA TCT GGA CCC TAC AAT GGC AGC [SEQ ID NO:49]

15

3' oligo (antisense) (2466)

AGCT AG ATC TGC CAT CCT ACT CGA GGG GCC AGC TGG [SEQ ID NO:50]

- The PCR product was then digested with the restriction enzyme BglII, to generate BglII ends to the PCR product. This 5' SOCS-1 PCR product, with BglII, ends was then ligated as follows: 20 3'SOCS-1 arm in pBgalpAloxNeo and 3'SOCS-1 arm in pBgalpAloxNeoTK, which had been linearized with the unique restriction enzyme BamHI. This resulted in the following vectors being formed:

5'&3'SOCS-1 arms in pBgalpAloxNeo

- 25 and 5'&3'SOCS-1 arms in pBgalpAloxNeoTK

These were the final SOCS-1 knockout constructs. Both these constructs lacked the entire SOCS-1 coding region (SOCS-1 EXON), being replaced with portions of the Bgal, B globin polyA, PGK promoter, neomycin and PGK polyA sequences. The 5'&3'SOCS-1 arms in 30 pBgalpAloxNeoTK vector also contained the thymidine kinase gene sequence, between the neomycin and PGK poly A sequences.

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The vectors: 5'&3'SOCS-1 arms in pBgalpAloxNeo  
and 5'&3'SOCS-1 arms in pBgalpAloxNeoTK  
were linearized with the unique restriction enzyme NotI and then transfected into Embryonic  
5 stem cells by electroporation. Clones which were resistant to neomycin were selected and  
analysed by southern blot to determine if they contained the correctly integrated SOCS-1  
targeting sequence. In order to determine if correct integration had occurred, genomic DNA  
from the neomycin resistant clones was digested with the restriction enzyme EcoRI. The  
digested DNA was then blotted onto nylon filters and probed with a 1.5Kb EcoRI /Hind III  
10 DNA fragment, which was further 5' of the 5'arm sequence used in the knockout constructs.  
The band sizes expected for correct integration were:

Wild type SOCS-1 allele 5.4Kb

15 SOCS-1 knockout allele 8.2Kb in 5'&3'SOCS-1 arms in pBgalpAloxNeo  
or 11Kb in 5'&3'SOCS-1 arms in pBgalpAloxNeoTK transformed cells.

Those skilled in the art will appreciate that the invention described herein is susceptible to  
variations and modifications other than those specifically described. It is to be understood that  
20 the invention includes all such variations and modifications. The invention also includes all of  
the steps, features, compositions and compounds referred to or indicated in this specification,  
individually or collectively, and any and all combinations of any two or more of said steps or  
features.

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Table 4.1

Summary of ESTs derived from mouse SOCS-4 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-4	Mouse	mc65f04	5'	EST0549700	d13.5-14.5 mouse embryo	m4.1
		mf42e06	5'	EST0593477	d13.5-14.5 mouse embryo	m4.1
		mp10c10	5'	EST0747905	d 8.5 mouse embryo	m4.1
		mr81g09	5'	EST0783081	d13 embryo	m4.1
		mt19h12	5'	EST0816531	spleen	m4.1

Table 4.2

Summary of ESTs derived from human SOCS-4 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-4	Human	27b5	5'	EST0534081	retina	h4.2
		30d2	5'	EST0534315	retina	h4.2
		J0159F	5'	EST0461188	foetal heart	h4.2
		J3802F	5'	EST0461428	foetal heart	h4.2
		EST19523	5'	EST0958884	retina	h4.2
		EST81149	5'	EST1011015	placenta	h4.2
		EST180909	5'	EST0951375	Jurkat T-lymphocyte	h4.2
		EST182619	5'	EST0953220	Jurkat T-lymphocyte	h4.1
		ya99h09	3'	EST0103262	placenta	h4.2
		ye70c04	5'	EST0172673	foetal liver/spleen	h4.2
		yh53c09	5'	EST0197390	placenta	h4.2
			3'	EST0197391		h4.2

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yh77g11	5'	EST0203418	placenta	h4.2
	3'	EST0203419		h4.1
yh87h05	5'	EST0204888	placenta	h4.1
	3'	EST0204773		h4.1
yi45h07	5'	EST0246604	placenta	h4.2
yj04e06	5'	EST0258541	placenta	h4.1
	3'	EST0258285		h4.1
yy12h06	5'	EST0309968	foetal liver spleen	h4.2
yy56a06	3'	EST0346924	foetal liver spleen	h4.2
yy60e02	5'	EST0347259	foetal liver spleen	h4.2
	3'	EST0347209		h4.2
yy92g03	5'	EST0355932	foetal liver spleen	h4.2
	3'	EST0355884		h4.2
yy97h06	5'	EST0357618	foetal liver spleen	h4.2
	3'	EST0357416		h4.2
yy90f01	5'	EST0372402	foetal liver spleen	h4.2
yt69c03	5'	EST0338395	foetal liver spleen	h4.2
	3'	EST0338303		h4.2
yy30a08	3'	EST0458506	foetal liver spleen	h4.2
yy55f07	5'	EST0465391	foetal liver spleen	h4.2
	3'	EST0463331		h4.2
yy57h09	5'	EST0464336	foetal liver spleen	h4.2
	3'	EST0458765		h4.2
yy87h02	5'	EST0388085	melanocyte	h4.2
yy98c11	5'	EST0400679	melanocyte	h4.2
	3'	EST0400680		h4.2
yy68d10	5'	EST0441370	placenta (8-9 wk)	h4.2
yy82a03	5'	EST0463005	placenta (8-9 wk)	h4.2

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	3'	EST0433678		h4.1
yx08a07	3'	EST0407016	melanocyte	h4.1
yx72h06	5'	EST0435158	melanocyte	h4.2
	3'	EST0422871	melanocyte	h4.1
yx76b09	5'	EST0434011	melanocyte	h4.2
yy37h08	5'	EST0451704	melanocyte	h4.2
yy66b02	5'	EST0505446	multiple sclerosis lesion	h4.2
za81f08	5'	EST0511777	foetal lung	h4.2
zb18f07	3'	EST0485315	foetal lung	h4.1
zc06e08	5'	EST0540473	parathyroid tumor	h4.1
	3'	EST0540354		h4.1
zd14g06	3'	EST0564666	foetal heart	h4.1
zd51h12	3'	EST0578099	foetal heart	h4.1
zd52b09	5'	EST0582012	foetal heart	h4.1
	3'	EST0581958		h4.1
ze25g11	3'	EST0679543	foetal heart	h4.1
ze69f02	5'	EST0635563	retina	h4.2
	3'	EST0635472		h4.1
zf54f03	5'	EST0680111	retina	h4.2
zh96e07	5'	EST0616241	foetal liver spleen	h4.2
	3'	EST0615745		h4.2
zv66h12	5'	EST1043265	8-9w foetus	h4.2
zs83a08	5'	EST0920072	germinal centre B cell	h4.1
	3'	EST0920016		h4.1
zs83g08	5'	EST0920121	germinal centre B cell	h4.1
	3'	EST0920122		h4.1

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**Table 5.1**  
**Summary of ESTs derived from mouse SOCS-5 cDNAs**

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-5	Mouse	mc55a01	5'	EST0541556	d13.5-14.5 mouse embryo	m5.1
		mh98f09	5'	EST0638237	placenta	m5.1
		my26h12	5'	EST0859939	mixed organs	m5.1
		vc24c06	5'	EST0819106	heart	m5.1

**Table 5.2**  
**Summary of ESTs derived from human SOCS-5 cDNAs**

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-5	Human	EST15B103	?	EST0258029	adipose tissue	h5.1
		EST15B105	?	EST0258028	adipose tissue	h5.1
		EST27530	5'	EST0965892	cerebellum	h5.1
		zf50f01	5'	EST0679820	retina	h5.1

**Table 6.1**  
**Summary of ESTs derived from mouse SOCS-6 cDNAs**

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-6	Mouse	mcc4c05	5'	EST0525832	d19.5 embryo	m6.1
		md48a03	5'	EST0566730	d13.5-14.5 embryo	m6.1
		mf31d03	5'	EST0675970	d13.5-14.5 embryo	m6.1
		mh26b07	5'	EST0628752	d13.5-14.5 placenta	m6.1
		mh78c11	5'	EST0637608	d13.5-14.5 placenta	m6.1
		mh88h09	5'	EST0644383	d13.5-14.5 placenta	m6.1



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mh94h07	5'	EST0638078	d13.5-14.5 placenta	m6.1
mi27h04	5'	EST0644252	d13.5-14.5 embryo	m6.1
mj29c05	5'	EST0664093	d13.5-14.5 embryo	m6.1
mp66g04	5'	EST0757905	thymus	m6.1
mw75g03	5'	EST0847938	liver	m6.1
va53b05	5'	EST0901540	d12.5 embryo	m6.1
vb34h02	5'	EST0930132	lymph node	m6.1
vc55d07	3'	EST1057735	2 cell embryo	m6.1
vc59e05	3'	EST1058201	2 cell embryo	m6.1
vc67d03	3'	EST1057849	2 cell embryo	m6.1
vc68d10	3'	EST1058663	2 cell embryo	m6.1
vc97h01	3'	EST1059343	2 cell embryo	m6.1
vc99c08	3'	EST1059410	2 cell embryo	m6.1
vd07h03	3'	EST1058173	2 cell embryo	m6.1
vd08c01	3'	EST1058275	2 cell embryo	m6.1
vd09b12	3'	EST1058632	2 cell embryo	m6.1
vd19b02	3'	EST1059723	2 cell embryo	m6.1
vd29a04	3'	? none found		m6.1
vd46d06	3'	? none found		m6.1

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Table 6.2

Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-6	Human					
		yf61e08	5'	EST0184387	d73 infant brain	h6.1
		yf93a09	5'	EST0186084	d73 infant brain	h6.1
		yg05f12	5'	EST0191486	d73 infant brain	h6.1
		yg41f04	5'	EST0195017	d73 infant brain	h6.1
		yg45c02	5'	EST0185308	d73 infant brain	h6.1
		yhl1f10	5'	EST0236705	d73 infant brain	h6.1
		yhl3b05	5'	EST0237191	d73 infant brain	h6.1
			3'	EST0236958		h6.2
		zc35a12	5'	EST0555510	senescent fibroblasts	h6.1
		ze02h08	5'	EST0603826	foetal heart	h6.1
			3'	EST0603718		h6.2
		z109a03	5'	EST0773936	pregnant uterus	h6.1
			3'	EST0773892		h6.1
		z169e10	5'	EST0683363	colon	h6.1
		zn39d08	5'	EST0718885	endothelial cell	h6.1
		zo39e06	5'	EST0785947	endothelial cell	h6.1

Table 7.1

Summary of ESTs derived from mouse SOCS-7 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-7	Mouse	mj39a01	5'	EST0665627	d13.5/14.5 embryo	m7.1
		vi52h07	3'	EST1267404	d7.5 embryo	m7.1

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Table 7.2

Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-7	HUMAN	STS W1-30171		(G21563)	Chromosome 2	h7.2
		EST00939	5'	EST0000906	hippocampus	h7.1
		EST12913	3'	EST0944382	uterus	h7.2
		yc29b05	3'	EST0128727	liver	h7.2
		yp49f10	3'	EST0301914	retina	h7.2
		zt10f03	5'	EST0922932	germinal centre B cell	h7.2
			3'	EST0921231		h7.1
		zx73g04	3'	EST1102975	ovarian tumour	h7.1

Table 8.1

Summary of ESTs derived from mouse SOCS-8 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-8	Mouse	mjl16e09	r1	EST0666240	d13.5/14.5 embryo	m8.1
		vj27a029	r1	EST1155973	heart	m8.1

Table 9.1

Summary of ESTs derived from mouse SOCS-9 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
	Mouse	me65d05	5'	EST0585211	d 13.5/14.5 embryo	m9.1

Table 9.2

Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-9	Human	CSRL-83f2-u		(B06659)	chromosome 11	h9.1
		EST114054	5'	EST0939759	placenta	h9.1

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yy06b07	3'	EST0434504	melanocyte	h9.1
yy06g06	5'	EST0443783	melanocyte	h9.1
zr40c09	5'	EST0832461	melanocyte, heart, uterus	h9.1
zr72b01	5'	EST0892025	melanocyte, heart, uterus	h9.1
	3'	EST0892026		h9.1
yx92c08	5'	EST0441160	melanocyte	h9.1
yx93b08	5'	EST0441260	melanocyte	h9.1
hfe0662	5'	EST0889611	foetal heart	h9.1

**Table 10.1**  
Summary of ESTs derived from mouse SOCS-10 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
	Mouse	mb14d12	5'	EST0549887	d19.5 embryo	m10.1
		mb40f06	5'	EST0515064	d19.5 embryo	m10.1
		mg89b11	5'	EST0630631	d13.5-14.5 embryo	m10.1
		mq89e12	5'	EST0776015	heart	m10.1
		mp03g12	5'	EST0741991	heart	m10.1
		vh53c11	5'	EST1154634	mammary gland	m10.1

**Table 10.2**  
Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-10	Human	aa48h10	3'	EST1135220	germinal centre B cell	h10.2
		zp35h01	3'	EST0819137	muscle	h10.2
		zp97h12	5'	EST0835442	muscle	h10.2

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	3'	EST0831211		h10.2
zq08b01	5'	EST0835907	muscle	h10.1
zr34g05	5'	EST0834251	melanocyte, heart, uterus	h10.2
	3'	EST0834440		h10.2
EST73000	5	EST1004491	ovary	h10.2
HSDHEX005	?	EST0013906	heart	h10.2

**Table 11.1**  
Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-11	Human	zt24b06	r1	EST0925023	ovarian tumor	11.1
		zr43b02	r1	EST0873006	melanocyte, heart, uterus	11.1
			s1	EST0872954		11.1

**Table 12.1**  
Summary of ESTs derived from mouse SOCS-12 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-12	Mouse	EST03803	5'	EST1054173	day 7.5 emb ectoplacental cone	m12.1
		mt18f02	5'	EST0817652	3NbMS spleen	m12.1
		mz60g10	5'	EST0890872	lymph node	m12.1
		va05c11	5'	EST0909449	lymph node	m12.1

**Table 12.2**  
Summary of ESTs derived from human SOCS-5 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-12	Human	STS-SHGC-13867			Chromosome 2	h12.2
		EST177695	5'	EST0948071	Jurkat cells	h12.1

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EST64550	5'	EST0997367	Jurkat cells	h12.1
EST76868	5'	EST1007291	pineal body	h12.2
PMY2369	5'	EST1115998	KG-1	h12.1
y638f04	5'	EST0108807	foetal spleen	h12.1
	3'			h12.2
yg74e12	5'	EST0224407	d73 brain	h12.1
yh13g04	5'	EST0237226	d73 brain	h12.1
	3'	EST0236992		h12.2
yh48b06	5'	yh48b06	placenta	h12.2
yh53a05	5'	EST0197282	placenta	h12.2
	3'	EST0197486		h12.2
yn48h09	5'	EST0278258	brain	h12.2
	3'	EST0278259		h12.2
yn90a09	3'	EST0302557	brain	h12.2
yo08f03	5'	EST0301790	brain	h12.2
	3'	EST0302059		h12.2
yo11e01	3'	? none found		h12.2
yo63b12	5'	EST0303606	breast	h12.2
	3'	EST0304085		h12.2
yq56g02	3'	EST0346935	foetal liver spleen	h12.1
zh57c04	3'	EST0594201	foetal liver spleen	h12.2
zh79b01	3'	EST0598945	foetal liver spleen	h12.2
zh99a11	3'	EST0618570	foetal liver spleen	h12.2
zo92h12	5'	EST0803392	ovarian cancer	h12.1
	3'	EST0803393		h12.2
zs48c01	5'	EST0925714	germinal centre B cell	h12.1
	3'	EST0925530		h12.2

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zs45h02	3'	EST0932296	germinal centre B cell	h12.2
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Table 13.1

Summary of ESTs derived from mouse SOCS-13 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-13	Mouse	ma39c09	5'	EST0517875	day 19.5 embryo	m13.1
		me60c05	5'	EST0584950	day 13.5/14.5 embryo	m13.1
		mi78g05	5'	EST0653834	day 19.5 embryo	m13.1
		mk10c11	5'	EST0735158	day 19.5 embryo	m13.1
		mo48g12	5'	EST0745111	day 10.5 embryo	m13.1
		mp94a01	5'	EST0762827	thymus	m13.1
		vb57c07	5'	EST1028976	day 11.5 embryo	m13.1
		vh07c11	5'	EST1117269	mammary gland	m13.1

Table 13.2

Summary of ESTs derived from human SOCS-13 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-13	Human	EST59161	5'	EST0992726	infant brain	h13.1

Table 14.1

Summary of ESTs derived from mouse SOCS-14 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-14	mouse	mi75e03	5'	EST0651892	d19.5 embryo	m14.1
		vd29h11	5'	EST1067080	2 cell embryo	m14.1

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vd53g07	5'	EST1119627	2 cell embryo	m14.1
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Table 15.1

Summary of ESTs derived from mouse SOCS-15 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-15	Mouse	mh29b05	5'	EST0628834	placenta	m15.1
		mh98b09	5'	EST0638243	placenta	m15.1
		ml45a02	5'	EST0687171	testis	m15.1
		mu43a10	5'	EST851588	thymus	m15.1
		my38c09	5'	EST878461	pooled organs	m15.1
		vj37h07	5'	EST1174791	diaphragm	m15.1
		AC002393			Chromosome 6 BAC	m15.1

Table 15.2

Summary of ESTs derived from human SOCS-15 cDNAs

SOCS	Species	EST name	End	EST no	Library source	Contig
SOCS-15	Human	EST98889	5'	EST1026568	thyroid	h15.1
		ne48b05	3'	EST1138057	colon tumour	h15.1
		yb12h12	5'	EST0098885	placenta	h15.1
			3'	EST0098886		h15.1
		HSU47924			Chromosome 12 BAC	h15.1



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